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Chemistry, Toxicity, and Bioavailability of Copper and Its Relationship to Regulation in the Marine Environment

Office of Naval Research
Workshop Report

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EXECUTIVE SUMMARY

Copper is one of the most ubiquitous contaminants found in many industrial and nonpoint source effluents, including discharges from Navy facilities and ships, into the marine environment. Discharge copper concentrations often exceed existing water quality criteria (WQC) or standards both in the effluent and the receiving systems. Regulatory compliance is often challenging because of the many copper sources and the adoption of very conservative water quality standards. In June 1997, invited speakers from the Navy “user” community, regulatory community, Office of Naval Research (ONR)-sponsored researchers, and other prominent scientists in the fields of trace metal speciation and ecotoxicity participated in a Workshop on Chemistry, Toxicity, and Bioavailability of Copper and Its Relationship to Regulation in the Marine Environment. The goal of the workshop was to improve our scientific understanding of copper in the marine environment and attempt to develop a solid scientific basis for future approaches to copper regulation. Nearly 20 presentations were made over a period of 2 days, at the end of which the “user-regulator” speakers and the scientific speakers met in two working groups to draft a set of recommendations.

The legal release and disposal of copper or copper-laden effluents into estuarine waters pose a serious environmental, engineering, and economic challenge to the Navy and to the country in general. This is because EPA-mandated WQC for copper effluents are at, or very close to, the ambient or “background” concentrations of copper in many estuaries, which are at the low $\mu\text{g/L}$ level. The treatment of copper-contaminated effluents to achieve such low levels, even if they are technologically attainable, may be cost-prohibitive, and may not be warranted, since there is little, if any, direct evidence in the natural environment of toxicity caused by small increases in copper levels.

The present EPA-mandated WQC justifiably fulfill their mission of protecting the environment, but do so from a relatively weak scientific basis and, most probably, are over-protective. A large body of scientific data indicates that it is the concentration of the “free” or aqueous cupric ion species ($\text{Cu(II)}_{\text{aq}}$) that relates best, but not exclusively, to the toxicity to marine organisms, particularly planktonic or larval forms. However, present WQC are based on concentrations of “total recoverable” copper and on unrealistic laboratory tests. The discrepancy between the current scientific knowledge and WQ standards tends to undermine confidence in the general applicability of the WQ standards.

The questions of copper speciation and bioavailability are being addressed indirectly by the regulatory community via the adaptation of a number of mechanisms, such as the use of dissolved copper concentrations rather than total recoverable copper and the use of Water Effects Ratios (WERs). However, these measures are, at present, an unsatisfactory substitute for realistic WQC/Standards based on bioavailability.

It was fully recognized by the workshop participants that the issues of speciation, bioavailability, and environmental impact of copper in estuaries and coastal waters are highly complex and will require much further study before they are satisfactorily resolved. At present, these studies should concentrate on (1) the development of standardized analytical methods of sampling and analysis, (2) the study of *in situ* physical and biological processes that may affect copper speciation, and (3) how toxicity effects on individual organisms ultimately translate into population and community effects.

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WORKSHOP GOALS

The Environmental Sciences Division of the Space and Naval Warfare Systems Center, San Diego (SSC San Diego)– formerly Naval Command, Control and Ocean Surveillance Center (NCCOSC) RDT&E Division (NRaD)– hosted an Office of Naval Research (ONR)-sponsored (6.2 Applied Research) Workshop on the Chemistry, Toxicity, and Bioavailability of Copper in the Marine Environment. The goal of the workshop was to clarify our scientific understanding of the nature, toxicity, and fate of copper in the marine environment to develop a better basis for future approaches to copper regulation. The objectives of the workshop were to bring Navy and regulatory representatives and scientific experts together to (1) define the current status and future direction of copper (Cu) regulations, (2) define problems and issues associated with the introduction of copper into the estuarine environment, and (3) discuss and evaluate the relationship between copper speciation, bio-availability, and toxicity. Although the copper workshop was highly focused and scientifically oriented, it concentrated on aspects of the problem that are relevant to Navy copper issues. An important and unique component of this first of two workshops was the bringing together of scientists and Navy users with environmental regulators, so that a cross-discipline understanding of engineering compliance constraints, scientific issues, and regulatory standards could be achieved.

BACKGROUND

Copper is a high-profile pollutant for the Navy and the nation because there are many sources releasing copper into marine or estuarine environments including storm waters, point sources, hull coatings, and discharges from commercial and Navy ships and facilities. Since laboratory studies have shown that copper is highly toxic to some marine organisms, the U.S. Environmental Protection Agency (EPA) has derived a low water quality criteria (WQC) for copper, namely 2.9 $\mu\text{g/L}$ (ppb) total recoverable copper. Recent U.S. EPA-proposed changes to the criteria would change the chronic value to 3.1 $\mu\text{g/L}$ dissolved copper and the acute value to 4.8 $\mu\text{g/L}$ dissolved copper. Many states have similarly low water quality objectives or standards. With the current regulatory approach, even lower copper levels may be mandated under regulatory programs. States are beginning to enforce National Pollutant Discharge Elimination System (NPDES) permits with low copper compliance limits that, if achievable, are cost-prohibitive and could negatively impact dry-dock operations, in-water hull cleaning, cooling water discharge, and potentially even freshwater discharges.

Currently, many effluents exceed copper standards and are generating more regulatory scrutiny. In some cases, mixing zones are being reduced or eliminated, thus making compliance difficult. This will result in higher compliance and remedial costs and potential delays in operations. The implementation of watershed pollutant management with enforced waste load allocation will add additional regulatory pressure to control copper inputs into harbors and estuaries.

A recent trend with the U.S. EPA and several states has been to take into consideration the differences in complexation capacity of regional waters by implementing the Water Effects Ratio (WER). This allows re-evaluation of toxicity tests by using local waters instead of clean laboratory water to determine the ratio between tests in the two media. If results permit, the ratio is then translated into a higher water quality standard for discharge permits. These trends reflect research done over the last decade including the ONR 6.1 Harbor Processes Program, which has demonstrated that copper toxicity is usually not closely related to the total concentration (Cu_T) that the regulations are based on, but rather the toxicity is based on dissolved labile or active ionic copper ($\text{Cu(II)}_{\text{aq}}$), which is at a much lower concentration in most environments. Since copper is rapidly complexed and rapidly bound to organic and inorganic moieties, it is less available to many organisms and thus, less toxic. Presently, these complexities are not being specifically considered in the regulatory discharge standards, even though the chemical and physical forms are known to relate directly to the toxicity to organisms. While the general issue of speciation is understood, there is a dearth of specific knowledge on how copper interacts with the marine environment and how quickly it does so. Thus, the workshop endeavored to discuss

1. The identification of the various forms of copper in Navy-impacted marine environments that are measurable with the presently available technology,
2. The known and suspected relationships among the various forms of copper and the copper buffering capacity of estuarine environments,
3. The known physical/chemical/biological conditions that are conducive to the formation and presence of the various identifiable forms of copper, and
4. The relationships between the various species of copper and their toxicity to specific groups of organisms.

WORKSHOP ORGANIZATION

The workshop took place at the Emerald Wyndham Hotel in San Diego over 2 days in early June 1997 and consisted of a series of individual presentations by invited speakers from the Navy “user” community, internationally known scientific experts, and representatives from the U.S. EPA. The audience consisted of all the speakers plus additional attendees from the Navy community, universities, and the EPA. The total number of attendees was kept small to promote discussion, and open debate was encouraged. On 3 June, after introductory comments by Dr. H. Guard and A. Lardis of ONR, the “user” and regulatory community presented material, and then the scientific experts presented summaries of pertinent Cu-related topics.

Twelve scientific presentations were made over the workshop’s 2 days and covered important chemical and biological aspects of copper in estuarine environments, beginning with its input from sources to its (final?) destination in the sediments and into marine organisms. The following topics were presented for discussion:

- Analytical Chemistry of Copper in Seawater
- Interactions of Copper with Estuarine Colloids
- Copper Speciation and Interactions with Biota
- Copper Bioavailability
- Effects of Copper on Marine Organisms

The presentations and their discussions lasted until noon of the second day. After a recess, all of the workshop participants were reconvened into either of two groups. Group 1 consisted of Navy users and regulators; group 2 was mainly scientists. The two groups met separately to draft a set of recommendations. It was possible to switch from one group to another at will. At the end of these sessions, the recommendations were presented to all the attendees by P. Seligman and A. Zirino of SSC San Diego for final discussion. At the end of the workshop, copies of visual aid materials (transparencies or viewgraphs) were collected from the individual speakers to facilitate the writing of this report. The final report was prepared from this material, from individual notes, and from material in the open scientific literature. The workshop program was as follows:

WORKSHOP AGENDA

ONR Workshop on Chemistry, Toxicity, and Bioavailability of Copper and Its Relationship to Regulation in the Marine Environment

June 3, 1997 (Agenda)

0800	Registration	
0830	Welcome and Introduction	H. Guard
0840	Meeting Objectives/Navy Copper Issues	P. F. Seligman
0855	Current and Future Trends in Metal Quality Criteria	C. Delos
0915	Copper Regulatory Status: Navy Perspective (Status of Water Effects Ratio)	R. Gauthier/P. Earley
0935	Navy User Discussion (Ship Issues) UNDS, AF Coatings, Hull Husbandry	G. Smith, B. Holmes, J. Bohlander, T. McCue
1020	Navy User Discussion (Shore Issues)	R. Benze, C. Chun

Cu Analysis and Speciation

1040	Copper in Seawater—Analytical Overview	A. Zirino
1110	Measurement of Activity and Complexation	K. Bruland
1210	Lunch	

Cu and Biota

1315	Cu and Estuarine Colloids	P. Santschi
	Cu: Phytoplankton and Bacteria (with related material from agriculture)	A. G. Lewis
1415	Copper and Larvae in S. D. Bay	C. Di Bacco and L. Levin
1515	Copper Speciation and Biota Interaction	J. Moffett
1615	Cu Bioavailability (Water/Sediments)	P. Paquin
1730	Adjourn	

June 4, 1997 (Agenda)

0815	Digestive Processes and Bioavailability	L. Mayer
0900	Trace Metal Effects on Benthos	S. N. Luoma
1000	S. D. Bay: DNA Damage in Marine Organisms	R. Streib-Montee and S. Steinert
1045	Developmental Toxicity in Fish	P. Weis
1145	Summary of Previous Day's Presentations	A. Zirino and P. F. Seligman
1300	Discussion: Navy User Issues, Needs, and Regulatory Status (Group I) Discussion: Science Gaps (Group II)	Navy User Group (Seligman lead) Scientists (Zirino lead)
1430	Summary Discussion and Recommendations	All Participants
1500	Adjourn	

SUMMARY OF PRESENTATIONS

INTRODUCTIONS

Dr. H. Guard, Acting Director of Biology and Biomedical Sciences Division and A. Lardis, Manager of the Environmental Quality Applied Research Program at ONR provided introductory remarks.

REGULATORY OVERVIEW AND NAVY USER GROUP PRESENTATION

WORKSHOP OBJECTIVES AND OVERVIEW OF COPPER ISSUES IN THE MARINE ENVIRONMENT (P. F. Seligman)

Copper is a nearly ubiquitous contaminant in Navy as well as industrial and urban discharges. There are numerous copper sources, many with no clear engineering pollution control solutions. From shore-based facilities, there are both point and nonpoint sources (e.g., shipyards and storm drains). Ships themselves are sources of copper from antifouling coatings, in-water hull cleaning, and liquid discharges (e.g., bilge, gray water, cooling water, etc.). An example of a source is the release of copper from antifouling coatings, where the metal is released at approximately 17 $\mu\text{g}/\text{cm}^2/\text{day}$, as measured from a dynamically exposed typical ablative copper coating. This release rate would introduce approximately 7,200 kg of Cu per year into San Diego Bay from Navy vessels, which is about 23% of the total estimated annual loading of copper into the bay.

Uniform National Discharge Standards (UNDS) for Federal vessels have been authorized to be jointly developed by the Navy and EPA. These standards, including those for copper, would apply nationally and have precedence over state standards. Although the UNDS take into account environmental effects, the practicality of pollution control options is a major factor in deciding whether marine pollution control devices will be implemented.

The goal of the Clean Water Act (CWA) is to maintain both human health and the integrity of biological communities in the receiving system. To ensure this goal, the current EPA approach to water quality criteria (WQC) as total recoverable Cu is intentionally overprotective:

Because of uncertainty concerning the relationship between the analytical chemistry and the toxicity of metals, aquatic life criteria for metals are expressed in terms of analytical measurements that result in the criteria providing more protection than necessary for the aquatic life in most bodies of water.

(Interim Guidance on Determination and Use of Water-Effect Ratios for Metals, U.S. EPA, 1994a).

Conservative water quality standards, which do not take copper bioavailability into account, are making compliance difficult or, in some cases, not attainable. Current trends in water quality regulation, however, are making allowances for the over-protection: (1) The copper WQC (U.S. EPA, 1995) are proposed to be changed to permit slightly higher concentrations, but even more important, the dissolved fraction, rather than total recoverable copper, would be used for measurements. (2) The watershed approach is being encouraged by EPA, which evaluates total contaminant loading into the receiving waters and attempts to balance allowable discharges through assigned “waste load allocation” of those discharges. (3) Site-specific water quality criteria can be developed by applying the

Water Effects Ratio (WER) or recalculation method using local species to evaluate the degree to which local waters reduce toxicity through complexation. (4) Ultra clean sampling and analysis techniques are being encouraged because they yield better accuracy and often produce lower measurement results than traditional sampling and analysis.

CURRENT AND FUTURE TRENDS IN METAL WATER QUALITY CRITERIA (C. Delos)

The 1995 Ambient Water Quality Criteria—Saltwater Copper Addendum (Draft) provides new values for WQC for copper (U.S. EPA, 1995). The new values were derived from additional toxicity data and are expressed as dissolved copper, rather than total recoverable of the existing 1984 WQC of 2.9 $\mu\text{g}/\text{L}$. The new criteria are defined as Criteria Continuous Concentration (CCC/Chronic) of 3.1 $\mu\text{g}/\text{L}$ and the Criteria Maximum Concentration (CMC/Acute) of 4.8 $\mu\text{g}/\text{L}$. Like the previous WQC, the new values are extrapolated to protect 95% of marine and estuarine species. It is anticipated that these values will become final in 1998. These new copper WQC have been promulgated in draft regulation, *Water Quality Standards; Establishment of Numeric Criteria for Priority Toxic Pollutants for the State of California* (U.S. EPA, 1997) to fill a gap that was created in 1994 when the courts overturned California's water quality control plans containing WQC for toxic pollutants. EPA recognizes that WQC are often over-protective because of the way they are calculated. Therefore, EPA has allowed for alternative methods of calculation and for the development of site-specific criteria.

Copper bioavailability issues are typically more significant in freshwater than marine environments. However, the Water Effects Ratio used to estimate bioavailability was successfully used in New York/New Jersey to raise the WQC by a factor of about 2, which allowed the treatment works to discharge without violation. The U.S. EPA is also supportive of the fish gill modeling approach being put forth by DiToro, Allen, and others (*vide infra*). This approach has been evaluated primarily in freshwater. This model correlates copper adsorbed onto the gills with later toxicity. Currently, EPA is not in favor of overriding chemical-specific criteria with whole effluent toxicity testing. For copper in salt water, one technical reason against sole reliance on Whole Effluent Toxicity (WET) testing is that there are no toxicity tests sensitive at the chronic criteria limit. Since copper is relatively slow-acting, the 24-hour averaging (CMC) is reasonable for defining the acute criterion. Chronic values (CCC) are set for 4-day averaging.

COPPER REGULATORY STATUS AND STATUS OF WATER EFFECTS RATIO: NAVY PERSPECTIVE (R. Gauthier and P. Early)

Regulatory Programs

There are at least three regulatory programs with which environmental managers at naval facilities must be concerned. The first program deals with discharge standards while the second and third programs are concerned with contaminated sediments. The first program, the National Pollutant Discharge Elimination System (NPDES), under the CWA, was developed to eliminate or reduce pollutant inputs to aquatic systems by imposing concentration and/or mass limitations on effluent discharges. Compliance with limits and/or water quality objectives is achieved through periodic monitoring of contaminants in effluents and ambient waters. Of the five most common metals in the NPDES programs across the U.S., copper has the most stringent limitation and monitoring requirements (U.S. GAO, 1996). The limits are usually adopted directly, and occasionally modified, from the federal WQC of 2.9 $\mu\text{g}/\text{L}$ total recoverable copper. A recent Addendum to the copper ambient WQC for saltwater (U.S. EPA, 1995) has changed the acute criterion to 4.8 $\mu\text{g}/\text{L}$ and the chronic criterion to 3.1 $\mu\text{g}/\text{L}$ dissolved copper. Figure 1 provides a cumulative probability distribution of

U.S. EPA acute laboratory derived copper toxicity values, which are compared to the proposed chronic and acute criteria.

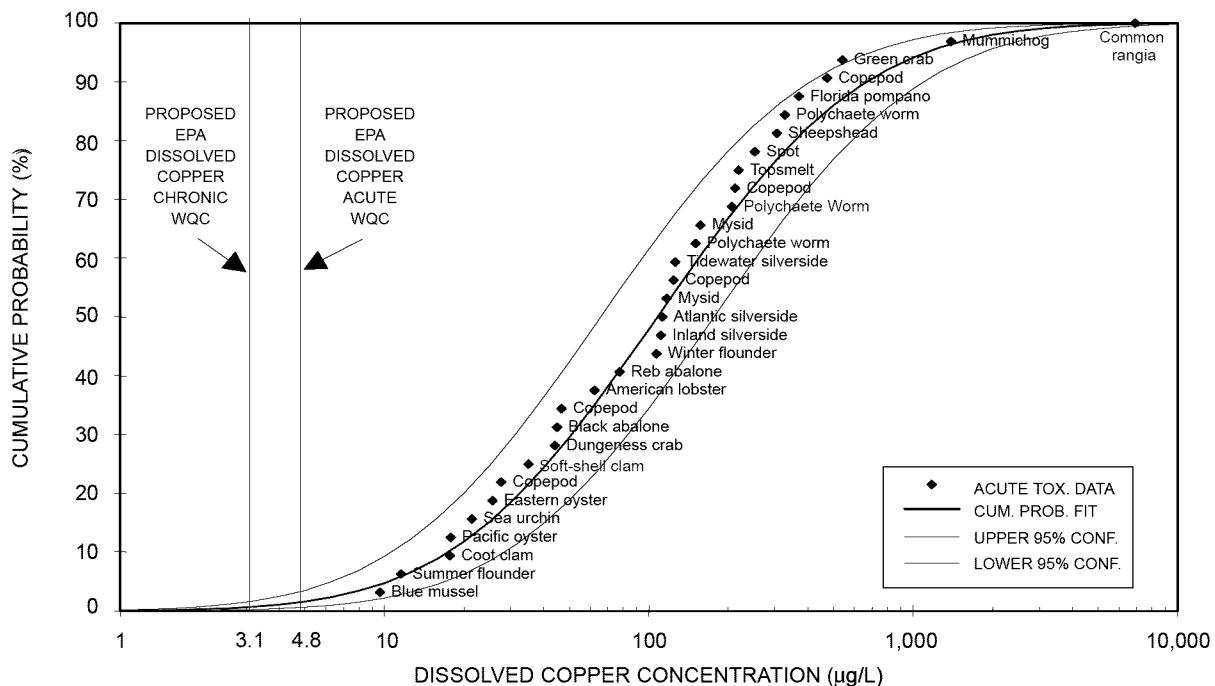


Figure 1. Acute copper toxicity probability distribution compared to proposed water quality criteria.

Regulatory provisions exist for the application of site-specific criteria, hydrodynamic mixing, and assimilative capacity to meet WQC, but there are relatively few successful implementations of these methods in marine or estuarine systems. Consequently, many NPDES permittees find it increasingly difficult to comply with their copper discharge regulations as regulators across the U.S. continue to impose more restrictive copper limits.

The second program, the Superfund Program, referred to by the Department of Defense (DoD) and the U.S. Navy as the Defense Environmental Restoration Program, requires facility operators to investigate potentially contaminated sites from past waste leakage or disposal practices to determine the need for remediation. An extensive environmental assessment is often performed in which contaminants of potential concern are measured in soil, sediments, and biota. Because copper has been a common Navy pollutant for many decades, copper can be found in these environmental media, adjacent to installations.

The U.S. EPA has no sediment quality criteria for copper; however, there are effects-based benchmark concentrations that have been used as screening levels in assessment and cleanup decisions: for example, the National Oceanographic and Atmospheric Administration (NOAA)-sponsored Effects Range-Low (ER-L, 70 µg/g) and Effects Range-Median (ER-M, 390 µg/g) values for copper (Long et al., 1995). This empirical data set was obtained from a set of aquatic effects studies (selected to meet certain data quality objectives by the authors) in which significant effects were observed in organisms that inhabited sediments containing the listed copper levels. The ER-L corresponds to the 10th percentile for this list, while the ER-M corresponds to the 50th percentile. Although this data set was never intended to serve in a regulatory context, it nevertheless is often used in helping risk man-

agers make environmental decisions about sediments containing certain levels of copper and other contaminants.

There are many approaches that have been used to assist risk assessors and managers in making decisions on copper-contaminated sediments. Two approaches are the Equilibrium Partitioning Approach and the Apparent Effects Threshold. The state of Washington has developed sediment management standards consisting of a set of both quality criteria and cleanup criteria, using a combination of these approaches. In the case of copper, Washington has the same value for both criteria and that value (390 $\mu\text{g/g}$, later revised to 270 $\mu\text{g/g}$) was obtained specifically from the ER-M. Additionally, background levels and reference site levels are also used in regulatory actions, with those representing a wide spectrum of copper concentrations. Finally, the presence of excess acid volatile sulfides, which serve to bind divalent metals, can be used in the regulatory decisions to begin to address the issue of the copper's bioavailability.

A third assessment method, established by the Florida Department of Environmental Protection (FDEP), is termed the Threshold Effects Level (TEL) and Probable Effects Level (PEL). From Jones et al. (1997):

Unlike the ER-Ls and ER-Ms, the TEL and PEL also incorporate chemical concentrations observed or predicted to be associated with no adverse biological effect (no effects data). Specifically, the TEL is the geometric mean of the 15th percentile in the effects data set and the 50th percentile in the no effects data set. The PEL is the geometric mean of the 50th percentile in the effects data set and the 85th percentile in the no effects data set. Therefore, the TEL represents the upper limit of the range of sediment contamination concentrations dominated by no effects data. For copper, this value has been defined as 18.7 $\mu\text{g/g}$. The PEL represents the lower limit of the range of contaminant concentrations that are usually or always associated with adverse biological effects. The PEL value for copper is 108 $\mu\text{g/g}$.

The fourth program, the Dredge Disposal Testing Program, was developed to certify that sediments are clean enough for aquatic disposal prior to them being dredged up for construction or maintenance purposes. A tiered-testing approach is required by the co-administrators of this program: the U.S. EPA and the Army Corps of Engineers. In addition to toxicity testing, sediments are often subjected to a suite of chemical analyses to investigate those contaminants that may be causing the toxicity. Copper, by weight, is one of the more toxic compounds that can be found in Navy sediments and is probably found in most Navy dredging testing programs. The regulatory actions associated with this program are complex, based on decision trees addressing the chemical and toxicity results performed for the study, and ultimately affect where the sediments may be disposed.

Scope of Copper Contamination: A National Perspective

To provide perspective to the copper problem in the marine environment, some observations can be made from reviewing several national environmental reporting and monitoring programs. A review of the Toxics Release Inventory for increases of total releases from 1988 to 1995 shows that copper (by mass) had the greatest increase in total releases during that time frame (from 14,500,000 kg to 19,000,000 kg). Although these involve releases to the air and ground, as well as water, one can see that copper is a significant pollutant problem nationally. It is interesting to note that the chemicals' and metals' industries had the largest decrease in releases during this same time period. A significant source of copper into marine waters—the leaching of antifouling hull paints—

was not even considered in this inventory. Another significant source across the nation—copper eroding from automobile brakes and corroding from copper piping—which contributes to stormwater, is also not included in this inventory. The U.S. EPA's National Sediment Quality Inventory shows that copper was found to represent a potential risk to aquatic life at more sampling stations (by absolute number and by percent of total) than any other compound measured. Of the 16,000 stations sampled for copper, 7,000 were assessed to present a potential risk.

In 1990, the geometric mean tissue residue for copper in mussels from 107 sites in the NOAA's National Status and Trends Mussel Watch Program was 9 $\mu\text{g/g}$ dry weight. Typical low effects levels in mussels are about 3 $\mu\text{g/g}$ wet weight, which would correspond to a dry weight of about 15 $\mu\text{g/g}$. Although this mean value is not above the effects level, copper does appear to be ubiquitously found in mussel tissue at a level that should warrant some concern. Since mussels are commonly used as biological integrators of ambient water quality, these data imply that the levels found in the mussels are due to elevated levels of copper present in the water column across the nation, either in the particulate or dissolved phase. Data for fish liver residues revealed copper present at all 43 stations sampled, from 6 to 118 $\mu\text{g/g}$.

In summary, it is clear that, across the nation, a substantial quantity of copper is being released into marine environments. Copper is present in various environmental matrices, including the water column, sediments, and biota. Copper was the most frequently elevated contaminant in sediments associated with stations determined to pose potential risk to marine organisms. Copper is a concern to the Navy because it is present in the water, sediment, and biota adjacent to naval facilities, and there are known past and present sources for this copper. The primary regulatory drivers for the Navy are the very low copper limitations for effluents in NPDES permits, and also the fact that copper is found in Installation Restoration (Superfund) site investigations where cleanup is a possible requirement.

Development of Site-Specific Criteria

Limits derived for metals are based on a national set of toxicity test data. These data are the foundation for all NPDES limits. The limits based on these data are often overly protective because they do not take into account the natural buffering capacity or bioavailability of the water nor do the national criteria take into account local species. Through the use of a Water Effect Ratio or Recalculation method (formerly called Indicator Species Procedure), a NPDES permittee is able to alter the national data set to match its discharge and its effects to the regional environment. Any changes made to the national data set apply only to the specific area studied. With the approval of EPA, results obtained from studies can be applied to permit limits instead of national criteria.

The rationale for conducting site-specific studies is to ensure that the WQC for a body of water are neither over- nor under-protective for the aquatic community. The national WQC were developed to protect the biological integrity of all surface waters in the U.S., and therefore, may require modification for specific sites (U.S. EPA, 1994a). The EPA's procedures for developing site-specific WQC are designed to consider two general factors that may make the national criteria inappropriate for a specific ecosystem. According to these guidelines,

Site-specific criterion derivation may be justified because species at the site may be more or less sensitive than those in the national criterion document, or because ... differences in physical and chemical characteristics of water have been demonstrated

to ameliorate or enhance the biological availability and/or toxicity of chemicals in freshwater and saltwater environments.

(U.S. EPA, 1994a)

Other factors that have an effect on this modification are the specific organisms that inhabit the local water body and the changing role of site water on the toxicity of a specific chemical. Three procedures have been developed to address these factors:

1. The Recalculation method, a mathematical calculation that uses only regional species habitation;
2. The WER method, a laboratory procedure that determines the site water effect by comparing test results between species in site and reference water; and
3. The Resident Species Procedure, which addresses both factors by generating an acute toxicity test database using resident species in site water (Dobbs et al., 1994).

The Resident Species Procedure tends to result in an unduly protective value for chemicals such as copper when there is a large database of acute toxicity data. Review of the 1984 ambient WQC for copper shows that the most sensitive saltwater species is the blue mussel (*Mytilus edulis*) that has an acute value of 5.8 $\mu\text{g/L}$, which, when divided by 2.0, is the source of the copper WQC value of 2.9 $\mu\text{g/L}$ (U.S. EPA, 1994a). There are several other species that have acute values within one order magnitude of the blue mussel.

Application of Site-Specific Criteria

Several studies throughout the nation have examined the application of WERs, Recalculation and Resident Species to provide regulatory relief. For most metals, the use of the Resident Species Procedure affords less than one order of magnitude change in the national criteria (Dobbs et al., 1994). Overall, the results from all of the WER and Recalculation studies increased the Cu WQC by an average of 25.35 $\mu\text{g/L}$ in favor of the permittees. However, most of these were in freshwater systems. Typically, saltwater WERs increases are lower, in the range of 1.5 to 5 $\mu\text{g/L}$.

Two studies were able to adjust the national WQC for copper in the marine environment from 2.9 $\mu\text{g/L}$ to 4.37 $\mu\text{g/L}$ chronic in Louisiana, and from 7.9 $\mu\text{g/L}$ to 15 $\mu\text{g/L}$ acute, in New York/New Jersey Harbor/Connecticut. New EPA draft guidance, which is projected to be promulgated in 1998, will, in effect, raise the WQC (U.S. EPA, 1995). This guidance establishes that "except where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration of dissolved copper does not exceed 3.1 $\mu\text{g/L}$ more than once every 3 years on the average and if the 24-hour average concentration does not exceed 4.8 $\mu\text{g/L}$ more than once every 3 years on the average."

A review of the literature for the acute toxicity of copper on 25 marine species indicates that while the values spanned a 1,320-fold range, the acute toxicity for more than half of the species was within a factor of 20 of the lowest criterion (Jop et al., 1995). The species at the lower end of this range, specifically the blue mussel larvae, are driving the national criteria on which permit limits are based. If this list can be reduced or changed based on the regional species composition, it can have a significant effect on the limits.

Water quality criteria are derived from a cumulative probability distribution of the final acute toxicity values in the laboratory tests selected by EPA. Protection of 95% of the species means that 95% of the Final Acute Values (FAVs) fall above the criterion for a given compound. In the case of cop-

per, since there were 20 test species, the lowest FAV represents the number used to derive the criterion: 5.8 $\mu\text{g}/\text{L}$. The final chronic value (FCV) or Criterion Maximum Concentration (CMC) is obtained with a similar calculation or by dividing the final acute value by the final acute-chronic ratio. This value for copper is 2.9 $\mu\text{g}/\text{L}$.

The national WQC may be inappropriate when applied to all receiving systems, due to site-specific interactions that may alter bioavailability (Parkerton et al., 1989). Several studies have begun to examine copper toxicity and bioavailability as an alternative means of solving compliance problems with discharges. These studies often found no observable effects of metals present in a number of aquatic environments at concentrations that exceed EPA's WQC (Allen, 1993). Several factors contribute to these observations. First, the criteria could be correct but the site-specific assessment of the regional ecology is not appropriately sensitive. A major goal of the WER process is to address this issue. Second, analytical errors may have resulted in reported numerical values that were much higher than what is actually present in the environment.

The EPA is addressing this issue with new publications and workshops that focus on trace metal sampling and analysis. Finally, WQC have been developed based on the concentrations of total recoverable metals present in the toxicity tests, rather than the bioavailable fractions of these metals. However, there is some resistance from regulators to accept analytical values expressed as dissolved metals because of a lack of an adequate aquatic toxicity database or translators to relate total recoverable metal in effluents to bioavailable metal concentrations in the receiving water (Allen, 1993). This problem is closer to resolution because regulatory agencies have begun to require permittees to collect data on the total recoverable and dissolved fractions of metals for use in a national database.

The database used to establish the national WQC standards for copper is large. Consequently, statistical calculations to alter the final value (i.e., criteria) using resident species will likely have a negligible effect. For example, if the blue mussel (*Mytilus edulis*) was removed from consideration, the next lowest toxicity value would then be applied to the WQC calculations to derive limits. In this case, the next most sensitive species would be the Pacific oyster (*Crassostrea gigas*), which has a reported value that is only 2 $\mu\text{g}/\text{L}$ higher. When permit calculations for freshwater systems are made, larger differences can be obtained because species that are not found in the discharge environment can be excluded because they would not be affected. When making calculations for marine copper concentrations, it is more difficult to exclude species because many of the species that are in the national database exist within most saltwater systems.

Another reason the toxicity values in the national database may be exceedingly protective is that the toxicity tests that form the basis of these criteria are usually performed using filtered water from an uncontaminated source (e.g., Narragansett Bay, Rhode Island for marine WQC). Filtered water is lower in metal-binding particulate and (possibly) in colloidal organic matter than typical ambient waters. Toxicity tests performed in filtered water may, therefore, overestimate the toxicity of metals that interact with particulate matter or colloidal organic matter under ambient conditions (U.S. EPA, 1994a). Bound metals are generally considered to be significantly less bioavailable and toxic than dissolved metals (U.S. EPA, 1994a). Thus, a lower proportion of the metal added to ambient waters would be present in a toxic form due to the binding capacity of dissolved organic and particulate matter contained in the receiving waters.

A WER study might be useful if NPDES permit limits were close to the WQC, or the limits were near ambient concentrations. If the limit was close to the WQC, a Recalculation or WER might alter this number. If the limit was well above the WQC, any number produced would likely still be lower

than that limit already in place. If limits were near ambient water conditions, these species-specific procedures would not matter. In fact, if ambient waters were greater than the WQC, the water body would be considered “impaired,” and there is a possibility that no discharge would be allowed under the nondegradation policy. Treating copper in effluent to meet Federal Water Quality Criteria is also an option to meet limits; however, it is often not feasible because of the high cost of treatment.

Scientific Issues

Several studies have begun to examine copper toxicity and speciation, particularly the separation of copper into the free (aqueous) cupric ion ($\text{Cu(II)}_{\text{aq}}$) and into various organic complexes. Reports of copper toxicity in natural environments are generally based on incomplete consideration of Cu(II) chemistry (Flemming and Trevors, 1989). In general, organically bound copper is less available and, therefore, less toxic to aquatic organisms. Meador (1991) has summarized several studies that have demonstrated that organic compounds have a strong ameliorating effect on copper toxicity. The EPA has recognized that “The cupric ion is highly reactive and forms moderate to strong complexes and precipitates with many inorganic and organic constituents of natural waters, e.g., carbonate, phosphate, amino acids, and humates, and is readily sorbed onto surfaces of suspended solids” (U.S. EPA, 1994b). When these complexes and precipitates are formed, the copper within them is either completely nontoxic or its toxicity is significantly reduced (Medor, 1991).

Because of the evidence that the free copper ion ($\text{Cu(II)}_{\text{aq}}$) is largely responsible for eliciting toxicity in aquatic organisms, it may be beneficial to determine the fraction of biologically available copper ($\text{Cu(II)}_{\text{aq}}$) through measurement of speciation and mixing and partitioning studies. Some studies, however, have indicated that copper is still toxic when it is bound to solids. It is conceivable that adsorbed copper would become available due to shifts in equilibrium in the gill environment as well as through ingestion of particles (Erickson et al., 1996). However, EPA believes the conservative approach that is used to determine dissolved metal toxicity mitigates for any toxicity that may be exhibited by the particulate fraction (U.S. EPA, 1993). According to the EPA, if the requirements of a WER, Recalculation, or Resident Species Procedure do not meet the specific requirements of the discharger, it is possible to propose an alternative study to determine site-specific criteria. This study must be scientifically defensible and approved by the local regulatory agency and the EPA. Screening tools can be used to measure near-real-time copper concentrations to evaluate actual copper loading, and a prediction can be made regarding exposure to resident biota. In addition, laboratory tests can help to determine the bioavailability of copper in the permittee’s discharge.

Contaminant modeling could be initiated to determine the mass balance of heavy metals within the discharge environment combined with partitioning studies to determine the relationship between dissolved and total recoverable metals. This modeling can help the permittee and the regulators determine the relative contributions of contaminants from outfalls and the predicted response. Inputs from the discharge, when corrected for background levels, could be an insignificant source in terms of both the overall input budget and the potential environmental impact.

Instead of attempting to develop site-specific criteria, it might be more cost-effective to (1) examine the bioavailability of copper in the area of the discharge, focusing on the toxic fraction and any biological species known to uptake that fraction; (2) estimate the copper loading in the area of the discharge with screening techniques focusing on the permittees discharges; and (3) investigate copper fate and effects. This effort may include conducting near- and far-field mixing studies or optimizing an existing hydrodynamic model to determine the expected dispersion, speciation, and final concentrations relative to all point and nonpoint source contributions of copper. Finally, the

contaminant partitioning and exchange processes that control ambient levels can be better described through contaminant transport modeling.

Conclusion

The application of a WER or Recalculation Procedure is useful when permit limits are near the WQC or when the discharge environment allows for removal of sensitive species from the data set. Several copper studies have been successful in achieving higher regulatory limits. However, marine copper limits that were reviewed are only 2 to 10 $\mu\text{g}/\text{L}$ above the new EPA 4.8 $\mu\text{g}/\text{L}$ WQC. NPDES permit limits above the Federal Water Quality Criteria most likely could not be significantly changed through the application of a WER, Recalculation, or Resident Species Procedure. In addition to improved best management practices and increased pollution prevention measures to reduce copper discharge, the permittee could (1) investigate copper loading from the discharges through the use of screening techniques, (2) determine bioavailability and toxicity associated with various forms of copper, and (3) perform site-specific studies on the fate and effects of copper discharged to the regional environment.

NAVY USER ISSUES (SHIPS): UNIFORM NATIONAL DISCHARGE STANDARDS (UNDS), ANTIFOULING COATINGS, AND HULL HUSBANDRY (G. Smith, B. Holmes, J. Bohlander, and T. McCue)

One of the major environmental issues facing the Navy is the development of Uniform National Discharge Standards (UNDS) for DoD ships. Copper is one of the principal contaminants of concern because of copper-based antifouling coatings and the high use of copper in piping systems aboard vessels. UNDS are being cooperatively developed by the Navy and the U.S. EPA. The first phase of this study is the identification of the specific discharges for which marine pollution control devices (MPCDs) will be required and was due February 10, 1998. The second phase will take 2 years and will set performance standards for those MPCDs that were selected in Phase 1. There will probably be some research and development needed to engineer MPCDs that will meet the performance standards. Over 50 discharges have been identified and are currently being sampled to evaluate contaminant loading. It is clear that copper is a significant component in many of these discharges. The largest contributor to copper loading in many heavily used harbors and estuaries is the copper from antifouling coatings. Performance standards will take into account practicability and cost and will assess the potential impact from the discharges.

The principal antifouling paints used by the Navy today are the ablative copper coatings. These coatings, which are 40 to 55% copper and approximately 20% zinc, erode when the ship is underway, and leach copper into the water column when the ship is docked. The Navy is moving toward condition-based maintenance to extend the period between dry dockings. Dry-dock intervals are currently scheduled for between 5 and 7 years, but the Navy plans to extend the intervals to 10 to 12 years. With the current coating systems, this would mean more underwater hull cleanings, and *in-situ* hull repairs, which would be a source of additional copper into the receiving system. This increase in potential sources for environmental discharge is perceived as a potential problem. The Navy is currently researching a number of nontoxic coatings and testing a few of the most promising paints including silicone-based coatings. Full hull tests on the silicone paints have been initiated on two ships. While these paints show some promise, they do foul and need to be cleaned at regular intervals. Based on performance and cost, it is unlikely the Navy will change from copper coatings in the near future unless new regulation forces the change. It has taken approximately 10 years to implement ablative copper into the Fleet, and it is likely that it would take another 10 years to fully implement a new

coating into the Fleet. However, research and development in new coatings technology will continue. The majority (70%) of commercial ship hulls still use tributyltin (TBT) coatings, which also contain approximately 30% to 40% copper.

The Navy spends approximately \$500M per year for propulsion fuel, and approximately \$30 to 60M a year is wasted because of fouling that causes hydrodynamic drag. Even though the number of ships in the Navy has declined from 550 to 342 in the last 7 years, fuel consumption has remained almost the same because of increased operational tempo. Clearly, effective antifouling coating systems that remain fouling free and reduce drag could save tens of millions of dollars a year. *In-situ* hull cleaning and maintenance are required at periodic intervals for most naval vessels. This activity can release copper and other contaminants into the receiving system. Additional sources include paint overspray during application; underwater hull cleaning; water washdown in the dry dock; and finally, sandblast dust during the removal process. Oncoming regulations and UNDS may require that pollution control devices be put in place to continue underwater hull cleaning. Currently, technology is being developed in remote automated cleaning technology with collection and treatment of waste water to reduce the copper loading to acceptable levels. This capability is in the research and development stage, and if it is shown to be cost-effective, may become operational within several years. There is a need to define both the target regulatory level of copper and to refine the pollution control technology to remove metals and other constituents from the waste stream to that target level. Cost-effective technology currently exists to remove the particulate copper with high volume ultra-filtration, but new technology is needed to remove relatively low concentrations of dissolved copper from high volumes of saltwater effluent.

The need to maintain operational readiness with practical and cost-effective antifouling coatings and hull husbandry must be emphasized. Coatings that require minimal and low-cost maintenance and in-water hull cleaning techniques that are effective and require minimal time are important to maintaining the Fleet's ability to respond to immediate operational requirements. There is need for the operational Navy to have well-defined analytical techniques for quantifying copper to respond to regulatory requirements, including oncoming UNDS standards.

SHORE ISSUES: THE COST OF CONTROLLING COPPER (R. Benze)

National Copper Criteria

The 1972 Clean Water Act (CWA) initiated the use of technology-based criteria. These criteria were based on available pollution control technology, rather than environmental science. The established end-of-pipe limits were based on categorical industry "best available technology." Copper limits were typically in mg/L (e.g., Metals Products and Machinery daily limit = 3.8 mg/L). The 1987 reauthorization of the CWA emphasized the use of WQC for NPDES permitting instead of technology-based permitting. The WQC are developed to protect 95% of all fish, invertebrates, and plants (i.e., chemical concentration causing no acute toxicity to 95% of the species). Marine criteria are based on toxicological data for approximately 25 saltwater species. The Ambient Water Quality Criteria document for Copper (U.S. EPA, 1984) recognizes that toxicity is largely related to Cu(II)_{aq}, which forms moderate to strong complexes, and that the proportion of copper present as Cu(II)_{aq} is low (<1% in eutrophic waters). Of all the toxicity tests that went into the copper criterion derivation, the lowest acute values were 7.8 µg/L for the Pacific oyster and 5.8 µg/L for Blue mussel embryos using a copper sulfate solution (U.S. EPA, 1984; Martin et al., 1981). This yielded a final acute value of 5.8 µg/L and a chronic value of 2.9 µg/L as total recoverable metal. Although the EPA criteria document acknowledges that toxicity is related to Cu(II)_{aq} and suggests that acid-soluble Cu (pH 1.5 to 2.0

with 0.45- μm filtration) would probably be the most relevant measurement, the document recommends that total recoverable method be used since there is no approved method for “acid-soluble” measurements. This highly conservative value and the measurement method have been translated into NPDES permits over the last decade.

Impact of Criteria

Even after permit writers consider dilution zones, total recoverable to dissolved translators and other factors, NPDES permit effluent limits for copper are typically in the low $\mu\text{g/L}$ range. A typical Navy shipyard with a daily maximum limit of 33 $\mu\text{g/L}$ and monthly average of 19 $\mu\text{g/L}$ (total recoverable) may still exceed guidelines because of the nature of the discharges. Figure 2 shows a record of actual discharges that were periodically in noncompliance by exceeding the permitted copper limit.

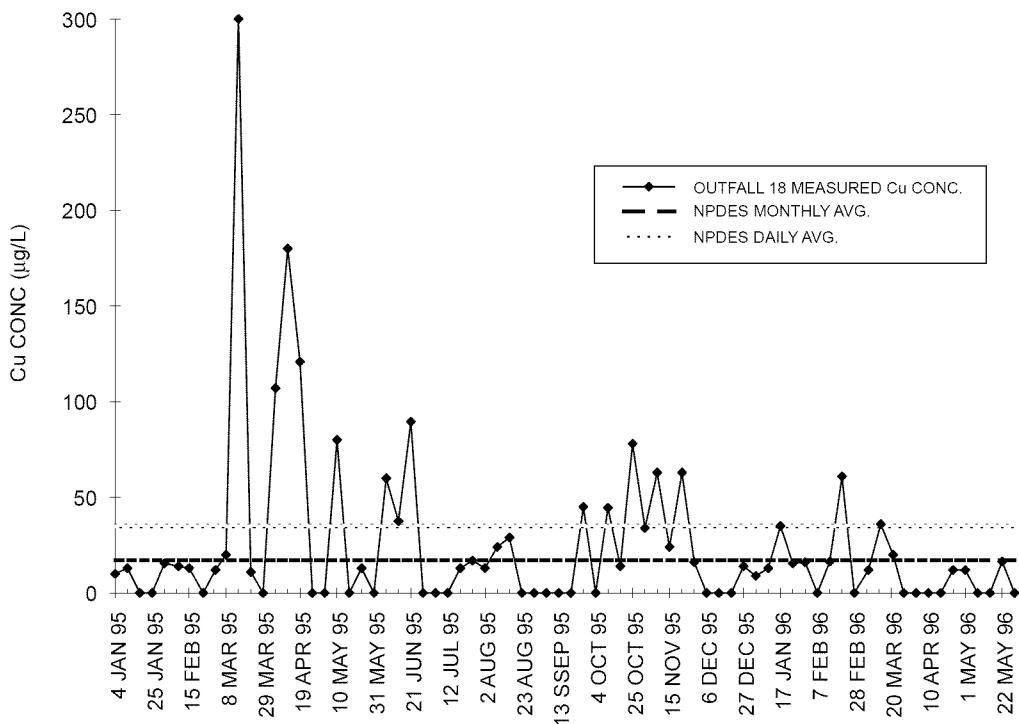


Figure 2. Final Puget Sound Naval Shipyard NPDES permit limits versus measured total Cu concentrations ($\mu\text{g/L}$).

In this example, investigations showed that the primary sources of copper were dust from hull or recycling work, from cutting, blasting, and chipping on or through copper antifouling paints in dry docks or in burn slabs. Studies also showed that copper spikes from the outfalls were almost exclusively in particulate form (figure 3). Sweeping dry docks alone is not effective. One study showed that approximately 80 kg of copper was left in a dry dock in the form of finely divided particulate left in the rough surface of the concrete after sweeping and recovered in a test using a vacuum cleaner. A proposed pollution control system that would modify all six dry docks to collect process water and stormwater is being implemented. The system would direct first flush 17,000 to 51,000 L of stormwater to the sewer, directing the remaining water into the embayment. The system would direct hull washwater, typically high in copper, to a collection system for processing.

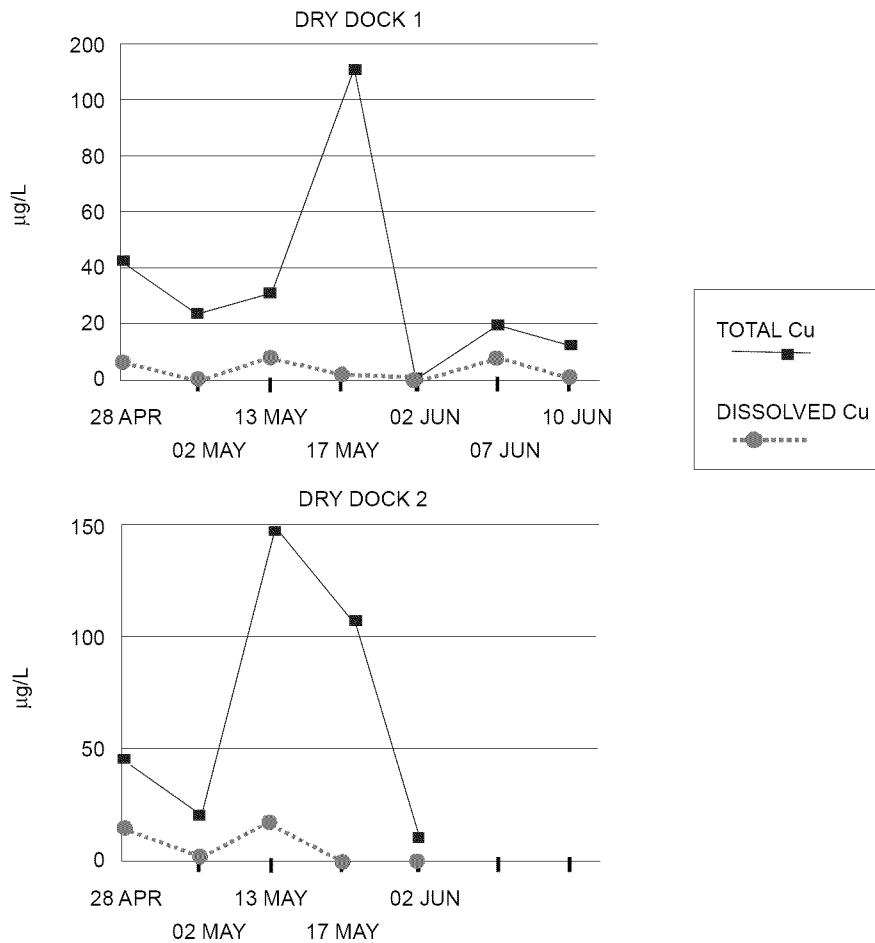


Figure 3. Comparison of particulate and dissolved copper in industrial effluents from Puget Sound Naval Shipyard (µg/L).

Costs

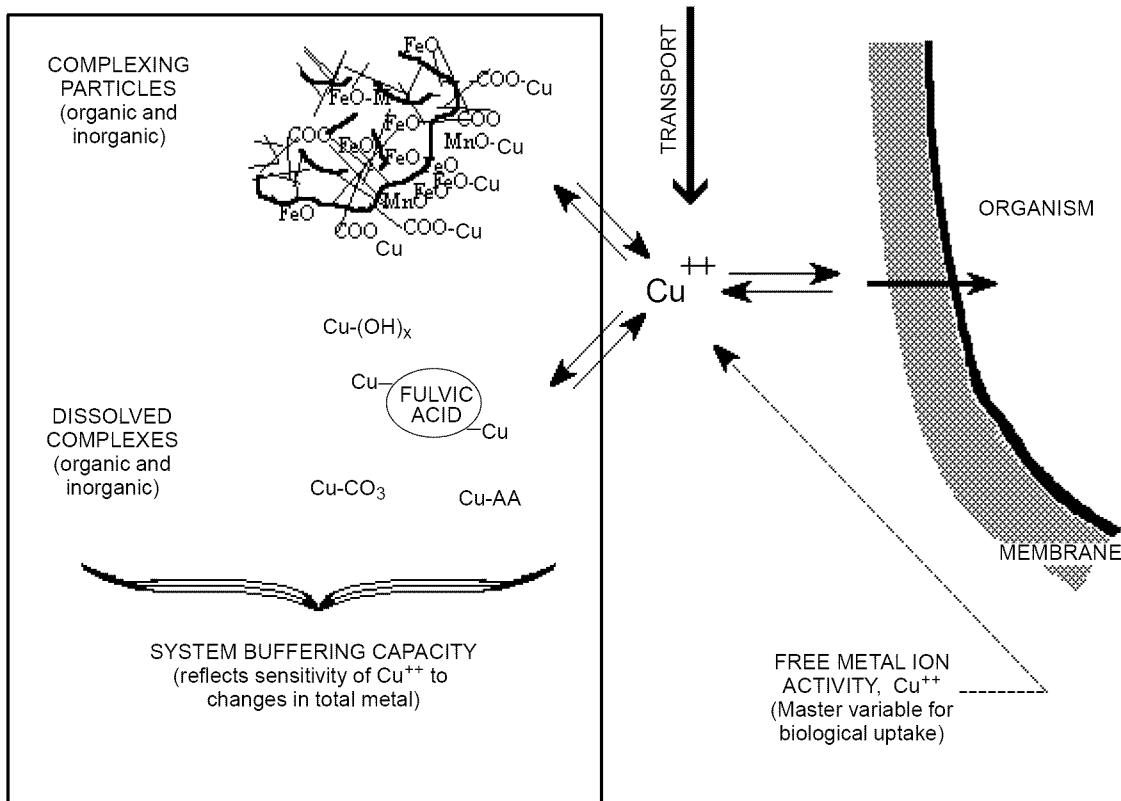
Currently, over \$2M is expended annually by the Puget Sound Naval shipyard in copper cleaning and containment costs, with tens to hundreds of thousands of dollars expended annually in sampling and analytical costs for permit-monitoring. Approximately \$3M will be expended to accomplish dry-dock modifications with additional costs anticipated to control copper in stormwater from non-dry-dock areas. Nationally, nearly every boatyard, shipyard, floating dry-dock operator or other activity that performs work on the hulls of ships has implemented, or soon will implement, some form of similar capability to control copper.

Other industrial activities that work with copper or copper compounds are also facing the need to install costly controls to meet NPDES limits derived from the national criterion. Stormwater is also coming under control. The overall annual cost of copper control in the U.S. is unknown but is undoubtedly in the area of hundreds of millions of dollars. In conclusion, seemingly small scientific discrepancies and conservatisms in the setting of national water quality criterion can result in nationally significant expenditures. The ratio of expenditures for the original copper toxicity testing to the national cost to achieve compliance, argues strongly for more comprehensive upfront science and testing to develop water quality criteria and to set NPDES discharge limits based on those criteria. This will ensure environmental protection but not costly overprotection.

SCIENCE GROUP PRESENTATIONS

THE ANALYTICAL CHEMISTRY OF COPPER IN SEAWATER (A. Zirino and K. Bruland)

A physico-chemical view of copper in seawater is given below (Buffe et al., 1990). It can be seen from figure 4 that copper in seawater may exist in various forms or chemical species, all of them exchanging copper with each other, at (presumably) various rates, with those species that exchange most rapidly being in steady-state equilibrium with each other. Table 1 lists some of the important forms of copper in seawater.



(after Buffe et al., 1990)

Figure 4. Copper species in seawater ($\text{Cu}^{++} = \text{Cu(II)}_{\text{aq}}$).

Table 1. Postulated physico-chemical forms of copper in seawater (under natural conditions).

Name of Species	Example
1. Hydrated Ion	$\text{Cu(II)}_{\text{aq}}$
2. Dissolved Inorganic Complexes	CuCl^+ ; $\text{Cu}(\text{SO}_4)^0$; $\text{Cu}(\text{OH})^+$
3. Dissolved Organic Complexes	
Labile	Cu-Glycine
Inert	$\text{Cu-(Humic Substance)}$
4. Colloidal Complexes	
Organic	$\text{Cu-Polysaccharides}$
Organic/Inorganic	Cu bound to organic matter adsorbed onto hydrated metal oxides
5. Particulate Copper	Cu adsorbed by fine clays retained on a $0.45\text{-}\mu\text{m}$ filter

Speciation

All of the species exchange with one another via $\text{Cu(II)}_{\text{aq}}$, and the exchange rates determine whether a particular copper species is in equilibrium with the bulk of the species under the particular experimental conditions. Under natural conditions ($\text{pH} = 8$), our experience with chemistry leads us to expect that $\text{Cu(II)}_{\text{aq}}$ interchanges freely and rapidly with the dissolved inorganic complexes and the labile organic complexes, and that most analytical methods could not differentiate between these species. The “inert” dissolved organic complexes are considered inert because they are not measurable directly by species-sensitive techniques such as those employed by electrochemists (e.g., voltammetry). Inert species include strongly complexed copper not directly reducible at a hanging mercury drop electrode (HMDE) or mercury film electrode (MFE) at an overvoltage sufficient to reduce (and thereby measure) the inorganic and labile organic complexes.

Cu-ethylenediaminetetraacetic acid (Cu-EDTA) is an example of this type of complex. Copper combined with humic substances would be considered kinetically inert (even though the copper is directly bound only to acetate) because the large size and predominantly organic (insulating) composition of the humic substance “molecule” would render it unmeasurable by the electrode at any practical overvoltage. Colloidal complexes, of whatever composition, would also be considered kinetically inert for the same reason. Therefore, we see that the terms “labile” and “inert” refer to the experimental conditions and instrumentation employed. They may also indicate a physical difference, *viz.*, species not in true solution would tend to be voltammetrically inert. Also, an otherwise labile $\text{Cu(II)}_{\text{aq}}$ ion would be rendered inert if adsorbed on a colloidal surface. Finally, it is commonly believed that essentially all species can be made labile if the organic material is destroyed with a strong oxidant and if the pH is lowered to <2 to dissolve Mn(IV) and Fe(III) hydroxy colloids, which can adsorb and trap Cu ion. Table 2 shows the distribution of copper species in San Diego Bay determined according to Zirino et al. (1998b).

Table 2. Typical distribution of copper species in San Diego Bay (Zirino et al., 1998b).

Species	pCu *	Conc. (µg/L)	Percent
Free Cu (Cu(II) _{aq})	11.4	0.00025	<0.01
Free Cu + Inorganically Complexed		0.0053	0.1
Dissolved, Organically Complexed		1.59	37.9
Colloidal (Largely Organic)		2.2	52.5
Particulate (>0.45 µm in size)		0.4	9.5

* pCu = $-\log_{10} (ACu(II)_{aq}) \cong -\log_{10} (Cu(II)_{aq})$

pCu, Buffering, and Complexation

It is quite explicit in figure 4 that Cu(II)_{aq} interacts with all forms, even algal cells. Analogously to pH, the inverse of the steady-state concentration of Cu(II)_{aq} on a logarithmic scale is known as pCu, which is approximately equal to $-\log_{10} (Cu(II)_{aq})$ and is used as a descriptor of the metal environment of the sample. The value of pCu depends on the number and types of organic and inorganic Lewis bases available to bind Cu ion in the sample. The resistance of pCu to changes in total copper ($\Delta pCu / \Delta \text{TotCu}$) also depends on the type and quantity of organic and inorganic Lewis bases available to complex Cu ion. This property is known as the Cu buffering capacity of the sample. The total amount of complexing ligand(s) and binding surfaces available is known as the copper complexation capacity of seawater (Buffle, 1988).

Methods and Instrumentation

Anodic Stripping Voltammetry. Voltammetry, already mentioned, is an electrochemical technique for measuring copper (and other trace metals) directly in seawater. It derives its remarkable sensitivity by reducing copper ion to form an amalgam on, or in, a solid mercury or mercury film electrode of small volume, under a potential gradient, over a period of time, and under conditions of high material flux, *viz.*, by stirring the sample or rotating the electrode. In one variation of the technique, known as anodic stripping voltammetry (ASV), the 1- to 15-minute pre-concentration step is followed by a “stripping” step in which copper in the amalgam is re-oxidized in solution by sweeping the potential, and the oxidation peak current is recorded as function of potential. Peak current is proportional to concentration and is the basis for quantitative analysis. In ASV, species can be differentiated by increasing the plating (reducing) potential to force dissociation of possible natural copper complexes (Cu-L), e.g.,



where L⁼ is the unassociated natural complexing agent, and thereby allowing the Cu⁺⁺ to be reduced. In some cases, it may also be possible to reduce the complex directly by applying a suitably high potential. It is also possible to discriminate on the basis of molecular size, because the rate of transport of the ion or molecule to the electrode is directly proportional to its diffusion constant. Thus, copper associated with large dissolved substances, such as humic and fulvic acids, yields very small peak currents and becomes unmeasurable at any practical potential. For the same reason, ASV is also insensitive to colloids. Finally, with thin Hg film electrodes on solid substrates, it is also possible to discriminate against complexed copper by rotating the electrode. Rotating the electrode limits the residence time of the complex at the surface of the electrode and prevents the reduction of Cu(II) produced by the natural dissociation of the complex.

Cathodic Stripping Voltammetry. In another, more sensitive variation of voltammetry known as cathodic stripping voltammetry (CSV), the process is somewhat different. In CSV, an organic complexing agent (R) is added to the sample to complex the available copper. Cu-R is then adsorbed on the surface of a mercury drop electrode (thin films don't work as well) under a potential gradient to concentrate it. Finally, the potential is swept to reduce the copper in Cu-R, and the resulting peak current is recorded. Species discrimination is obtained by the choice and amount of R in the sample. An organic ligand R with a great affinity for Cu (with a large stability constant) which can outcompete any natural ligand L, will cause the measurement to yield "total" values for Cu in the sample. On the other hand, a ligand R that can only outcompete the weak natural ligands in the sample will yield just a partial concentration that represents free Cu and inorganic Cu, plus Cu weakly bound to organic ligands. This process may be represented by



where CuL' represents the copper still bound to strong ligands.

This ability to shift the equilibrium to yield a measurable quantity gives rise to what is generally known as the "detection window," which depends on the type and quantity of reagent added, as well as the analytical signal generated by the adsorbed complex. Electrochemical speciation techniques have been reviewed recently by Mota and Correia dos Santos (1995).

Graphite Furnace Atomic Absorption Spectroscopy (GFAAS). GFAAS, the EPA-approved technique, is not sufficiently sensitive to measure copper directly in any but the most contaminated estuarine waters. The method consists of measuring the light absorption of excited Cu atoms brought to high temperature in a graphite furnace. Seawater is generally not analyzed directly because of background interference caused by salt, although in the early days of the technique, "matrix modifiers" were employed to ease this problem. Presently, copper in the sample must first be isolated by using a separation technique, and then the copper in the saltless concentrate is atomized in the graphite furnace. Common copper isolating techniques include (1) retaining Cu on ion exchange columns, such as Chelex 100 or immobilized hydroxyquinoline; (2) using co-precipitation methods; or (3) complexation with a strong complexing agent, such as ammonium pyrrolidine dithiocarbamate (APDC), followed by extraction into a solvent such as chloroform, MIBK, or Freon (Bruland et al., 1985). Speciation by GFAAS is limited, since the pre-concentration techniques are designed to completely shift the equilibrium toward the added reagents. Thus, GFAAS can be considered a measurement that approaches the total copper concentration, although Cu "buried" in unreactive matrices may be missed. Details of nonelectrochemical speciation techniques may be found in reviews by Apte and Batley (1995) and Marshall and Momplaisir (1995).

Copper Ion Selective Electrode. Several recent papers have proposed the use of the Orion 90-24 (jalpaite) copper ion selective electrode in seawater (Zirino et al., 1998a and references therein). This electrode, when calibrated against seawater copper activity standards, is capable of reaching meaningful steady-state potentials in estuarine waters. By definition, the electrode measures the steady-state activity of $\text{Cu}(\text{II})_{\text{aq}}$ or $-\log_{10}(\text{ACu}(\text{II})_{\text{aq}})$. However, at this point, we will not linger on the definition of activity (A) and will consider it to be approximately equal to concentration. It is able to do this at very low concentrations of $\text{Cu}(\text{II})_{\text{aq}}$ because the measured Cu(II) resides at the surface of the electrode, and it reaches equilibrium with the ligands in solution. Presumably, the ligands that control Cu activity at the surface of the electrode are also controlling it in the bulk solution. This electrode measures only one species, the aqueous copper ion, and it even discriminates between $\text{Cu}(\text{II})_{\text{aq}}$ and inorganic forms such as CuCl^+ . Speciation can only be effected by converting the various copper

species present in solution to $\text{Cu(II)_{aq}}$. Total copper can be measured only after strong oxidation of the sample followed by acidification.

The Copper Complexation Capacity of Seawater

A parameter of first importance in toxicity studies is the complexation capacity of seawater, as defined previously. This is the available Lewis base that would complex an input of Cu ion and perhaps reduce its toxicity. Appropriately, this parameter is measured by titrating the sample with copper ion and then measuring the resulting “free ion” by voltammetry or with the ion selective electrode. Figure 5 shows a simulated seawater titration.

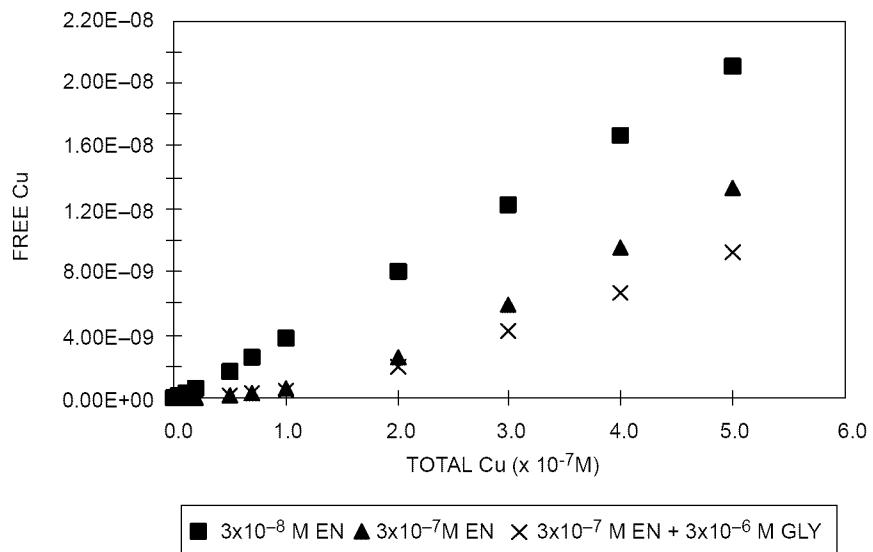


Figure 5. Simulated copper titration of seawater with added ethylenediamine (EN) and glycine (GLY).

The figure shows a plot of $\text{Cu(II)_{aq}}$ (Free Cu) against the total copper concentration (Total Cu) resulting from the copper additions to artificial seawater, under thermodynamic equilibrium conditions. Three curves are shown. The first describes the plot made when $3 \times 10^{-8} \text{ M}$ ethylenediamine (EN, a moderately strong copper complexing agent) is titrated. The curve shows a very weak break near $3 \times 10^{-8} \text{ M}$ total Cu, as expected. However, the break is probably not experimentally detectable. The second curve shows the plot of $3 \times 10^{-7} \text{ M}$ ethylenediamine. This curve shows a clear break at $1.5 \times 10^{-7} \text{ M}$ total copper. The break in the curve occurs because $\text{Cu(II)_{aq}}$, the free ion, is initially suppressed by complexation with EN, and then “breaks through” as the added copper ion exceeds the available EN. However, the “break” is not a quantitative measure of the added ligand. The third curve plots the data from a titration in which an additional $3 \times 10^{-6} \text{ M}$ glycine (GLY) (a weak copper ligand) has been added to the original $3 \times 10^{-7} \text{ M}$ ethylenediamine. The plot shows no discernible change in “break,” but the slope of the line beyond the break is decreased. The addition of glycine cannot be detected from the break.

The nonlinearity that occurs upon adding ligands takes place because each ligand in solution, competes for the “free copper ion” proportionately, in accordance with the product of its concentration and stability constant. The titration is quantitative and produces a sharp break when there is only a single, strong (large stability constant) copper-binding ligand in solution. When there is a mixture of potentially titratable ligands in solution, the position of the break, located by extrapolating the

curves, is no longer quantitative, but qualitative—an empirical measure of the total binding capacity of the water.

Stability Constants and Ligand Concentrations

Conditional stability constants (K_{Cond}) and copper-binding ligand concentrations (C_L) can be obtained by transforming copper titration data to a function that plots linearly when plotted against free Cu plus inorganic Cu, the signal obtained from voltammetry (Coale and Bruland, 1988):

$$[\text{Cu}']/[\text{CuL}] = ((1/[L]) \times [\text{Cu}']) + (1/([L] \times K_{\text{Cond}})) \quad (3)$$

where $[\text{Cu}'] = \text{Cu(II)}_{\text{aq}} + \text{inorganic copper complexes}$, and $[L] = \text{the concentration of complexing ligand obtained from the titration}$.

The model used in the transformation presupposes relationships of the type shown in Eq. 1. When $[\text{Cu}']/[\text{CuL}]$ is plotted against $[\text{Cu}']$ on the x-axis, the slope of the resulting line yields $(1/L)$, and the intercept yields K_{Cond} . It is obvious from Eq. 3 that K_{Cond} is not independent of L and that L must be known precisely in order for K to be determined. In fact, there exists an experimentally verified relationship that shows that L is inversely related to K (e.g., large L , smaller K_{Cond} and vice versa). It is not clear that L can be separated from K_{Cond} with data from natural samples in which the nature and amount of copper-binding matter is not known, using any titration or ligand competition technique. Furthermore, there is also more than a possibility that the organic matter in seawater possesses a statistical distribution of binding ligands with statistically distributed binding constants and may not lend itself to the analytical treatment described above (Buffe, 1988). If we add to this conceptual problem the ambiguities caused by different methods producing differing values of K and L because of different detection windows, we come to the conclusion that K_{Cond} and L must simply be regarded as empirically defined measures of the copper-binding capacity of seawater. Table 3 shows a sampling of K_{Cond} and L values obtained by various workers for different types of marine waters, using different techniques. Table 3 shows that a great deal of variability exists among the measured values that might reflect differences in methodologies as well as different environmental conditions.

Table 3. Conditional stability constants and copper-binding ligand concentrations for different types of marine waters.

Water Type	$L(\text{nM})$	K_{Cond}	Method	Reference
Open ocean	1.8	11.5	DPASV	Coale and Bruland, 1988
		11.8	Ligand Comp.	Hirose et al., 1982
	53 to 65	7.9 to 8.4	DPASV	Kramer, 1986
		13.2	Ligand Comp.	Moffett et al., 1990
	11	12.2	CSV	van den Berg, 1984
	36	9.80	MnO_2	Buckley & van den Berg, 1986
	135	8.57	DPASV	Buckley & van den Berg, 1986
	13.8	13	CSVA	Campos & van den Berg, 1994
Coastal	33 to 8.5	8.3 to 10.9	DPASV	Muller, 1996
	2.8	12.5	Chemiluminescence	Sunda and Huntsman, 1991
	5	13.1	Lig. Comp. + ASV	Scarano et al., 1992
	5	>12	Bioassay	Sunda and Ferguson, 1983
	3.8	12.3	Ligand Comp.	Sunda and Hansson, 1987

Table 3. Conditional stability constants and copper-binding ligand concentrations for different types of marine waters. (continued)

Water Type	L(nM)	K _{Cond}	Method	Reference
Estuarine	13	13.5	Lig. Comp. + ASV	Donat et al., 1994
	13 to 196	11.4 to 13	DPCSV	Apte et al., 1990
	78	10.5	CuISE, ASV	Zirino et al., 1998
	32	12.2	Ligand Comp.	Sunda and Hansson, 1987

Measurement of the Free Copper Ion Activity of Seawater

As is evident from figure 4, the free copper ion activity (expressed as pCu) is a thermodynamic measurement of the Cu ion “pressure” of the system. Activity (which is unitless) is different from concentration in that it represents a steady-state concentration of Cu ion that is relatively invariant with time until the buffer capacity of the system is consumed. An activity is analogous to a partial pressure. For example, we are familiar with the partial pressure of oxygen of 20.8%. Happily for us, this steady-state value is maintained constant (buffered) by the biota and the large reservoir of oxygen on the earth’s surface and is not likely to change dramatically until the entire buffering system is used up.

There have been several attempts to measure the Cu ion activity of seawater, both directly and indirectly. At present, the only direct method for measuring Cu activity in seawater is by using the Orion 90-24 (jalpaite) electrode (see section on Measurement of the Free Copper Ion Activity of Seawater). This method has just been introduced, however, and has not been fully tested in natural environments. Indirect techniques include direct voltammetric titration and voltammetry with ligand exchange, chemiluminescence combined with ligand exchange, and bioassay. The direct voltammetric technique computes pCu from stability constants and ligand concentrations computed from the results of a metal titration, and is subject to the errors discussed previously. Both voltammetric techniques and ligand exchange techniques using another type of signal (such as chemiluminescence) may introduce errors by displacing the Cu ion from weak natural ligands that almost certainly are present in seawater, thereby giving lower pCu values. All of the methods are calibrated against model Cu-ligand solutions of known composition. Because pCu relates directly to the toxicity of copper (*vide infra*), there is a great need to standardize its measurement.

THE INTERACTION OF COPPER WITH ESTUARINE COLLOIDS (P. H. Santschi)

Colloids, “particles too small to be resolved with an ordinary light microscope and which fail to settle out of solution...” (*Webster’s Collegiate Dictionary*, 1977), may play a very important role in the speciation of copper in seawater. Marine colloids are composed of microparticles and macromolecules, are from 1 nm to 1 μ m in size, and are composed primarily of polysaccharides (50%), proteins (10 to 20%), humic materials (10 to 20%), lipids (<5%), and mineralitic particles (1%). Marine colloids are also polymeric, polyfunctional, polydisperse, internally and externally porous, and hydrated to a variable degree. Once lumped in with “dissolved” organic matter, they have been recognized as an important, distinct component of such dissolved matter. The colloids’ source is primarily biological (macroalgae, phytoplankton, bacteria) with the inorganic component coming from hydrated metal (Fe-Mn) oxides and very fine clays. In seawater, the marine colloids act as Lewis bases, potentially binding copper with carboxylic ($-COO^-$), amino ($-NH_2$), and (possibly) sulphydrilic ($-S^-$) functional groups.

Early work on the speciation of copper (Florence and Batley, 1977) indicated that a large fraction of the total copper in estuarine samples was in the form of organic colloids or pseudocolloids (copper adsorbed on colloidal matter). Recent work in Prof. Santschi's laboratory at Texas A & M University at Galveston has confirmed those initial observations (Guo et al., 1995; Guo et al., 1996). Using atomic fluorescence microscopy in Prof. Buffle's laboratory in Geneva, Switzerland, Santschi and colleagues have identified polysaccharide fibrils of 1- to 2-nm diameter and 100- to 1000-nm length in seawater. These fibrils are believed to be the precursors of larger organic colloids that form *in situ* through aggregation. Using an ultraclean cross-flow ultrafiltration technique, they determined that colloidal copper constituted between 65 and 85% of the "dissolved" copper in Galveston Bay (Wen et al., 1996).

What are the consequences of the presence of colloidal forms of copper in solution? Basically, because of the large size of the colloids, the effective diffusion constant of copper is decreased over that of copper in the ionic form, which results in slower rate constants, slow sorption kinetics, and lower bioavailability (*vide infra*). Also, because of the heterogeneity of the colloidal functional groups to which copper is bound ($-COO^-$, $-O^-$, $-NH_2$, $-S^-$), Cu-ligand interactions are best described by statistical distribution functions rather than by individual constants (Buffle, 1988; Turner, 1995). The effect of the heterogeneity of binding sites affects the determination of the complexation capacity. In a copper titration, the strongest binding sites (largest K_{Cond} 's) are titrated first. Since the end of a copper titration is rather arbitrary, this has the effect of producing K_{Cond} 's that are inversely proportional to L , the calculated ligand concentration, as predicted by Perdue and Lytle (1983).

Typically, in an estuarine environment, colloids are continually produced through the excretion of polysaccharide fibrils by marine plants and aggregate and/or are adsorbed on larger molecular weight colloids (macromolecular carriers) and ultimately particulates, which would ultimately end in the sediments. These fibrils were also the transport vehicle for plant-produced ligands, such as phytochelatins, out of true solution and ultimately onto particulate surfaces. This "conveyor belt" to the sediments obviously serves as a major detoxifying process in that it takes copper out of solution, although Santschi pointed out that colloidally bound trace metals, and by extension, copper, can be taken up directly by penaeid shrimp around the gills and accumulated in the hepatopancreas.

COPPER INTERACTIONS WITH BIOTA (A. G. Lewis, J. Moffett, C. DiBacco, and L. Levin)

Depending on its concentration, copper can be either an essential nutrient or a toxin to marine organisms. There is no published evidence that copper has ever been limiting to growth in estuarine waters, but there is significant literature on its toxicity to many diverse types of marine organisms, including micro and macroalgae, cnidarians, annelids, crustaceans, molluscs, and fish under laboratory conditions (figure 1). However, direct copper toxicity in the environment has yet to be demonstrated conclusively. In part, this is because copper-sensitive species have ceased to exist in polluted environments. Thus, a reduction in species diversity is an expected effect—but, it is almost impossible to attribute the effect to copper *alone*, since most polluted environments receive a multiplicity of toxic elements and substances. There are, however, unique environments in which copper is the principal pollutant. Lake Orta, in northern Italy, is a good freshwater example. San Diego Bay may also be unique in this aspect, with a high density of copper-painted vessels and little freshwater input or industrial contamination.

Detrimental effects of copper to bacteria, phytoplankton, and zooplankton have been linked to the activity or concentration of $Cu(II)_{aq}$ in solution. (At constant ionic strength, activity and concentration

are related by a constant). Figure 6 shows the relationship between the survival of the diatom *Thalassosira pseudonana* and pCu, the negative logarithm of the copper activity. The good relationship between pCu of the various media and survivorship suggests that the pCu of the media is in equilibrium with the internal pCu of the cell and that the type of ligand used to fix the pCu in the cell is irrelevant. It also suggests that equilibrium is established rapidly across the cell wall and cell membrane.

Figure 6 is typical of many tests that use cultures of microorganisms and that have shown serious deleterious effects below pCu = 10.5 or so (Sunda, 1994). It is interesting to note that the range of copper concentration in estuaries is approximately 0.1 to 10 $\mu\text{g/L}$ or pCu 7 to 9, totally toxic to microalgae if it were not bound to organic matter. Not unexpectedly, copper has been found to be more toxic in oligotrophic waters than in eutrophic waters, and this effect can be attributed directly to the enhanced complexation capacity of eutrophic waters. Tolerance to copper is expected to vary from (biological) species to species and during the life history of a species. For zooplankton, there may be as many as 12 stages in a life cycle (plus embryo or egg).

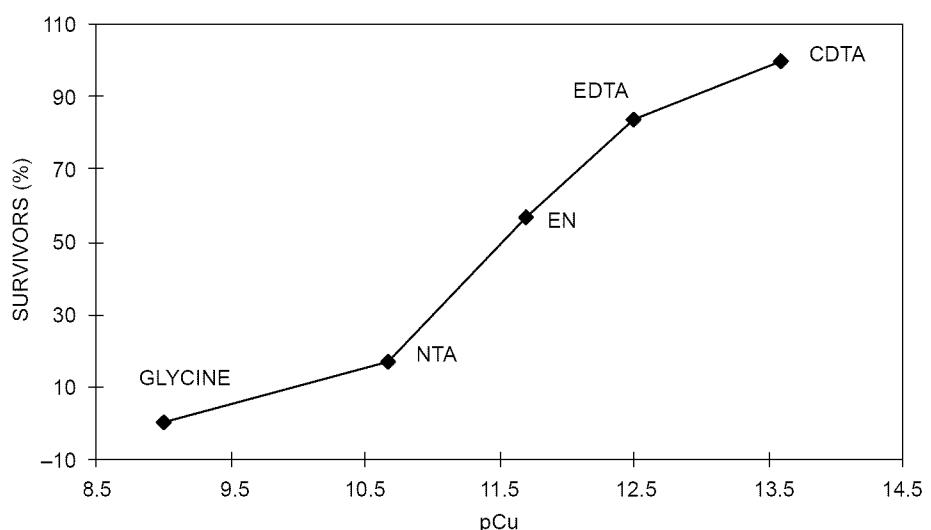


Figure 6. Relationship between survivorship and measured pCu for *Thalassosira pseudonana* in seawater media. Labels denote ligands used to adjust Cu(II)_{aq} at constant copper concentration.

This means that in order to evaluate the impact of copper on a biological species, it is necessary to know which stage is least tolerant. Also, some organisms are inherently highly tolerant to stress, including that from trace metals. This suggests that “natural” tolerance must be considered in the selection of bioassay organisms.

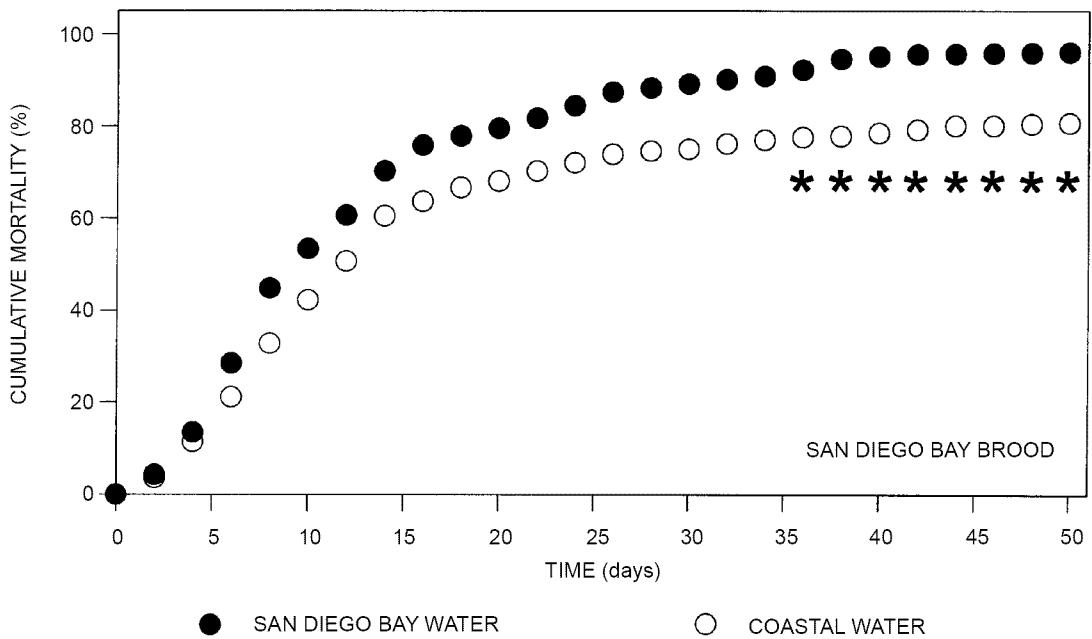
Tolerance to copper in marine organisms can occur through exclusion or through intracellular detoxification mechanisms. For example, under stress, phytoplankton may exude into the water metal-binding compounds that bind copper ion extracellularly, or they may produce complexing agents on the cell wall (exclusion). Conditional Cu-binding stability constants of exuded copper complexing ligands have typical values between 10^{-7} and 10^{-13} , comparable to the stability constants for “natural” organic ligands found in seawater. Despite their apparent importance, these exudates have yet to be identified. Phytoplankton may also control Cu(II)_{aq} activity internally by producing phytochelatins in response to copper stress. Cyanobacteria such as *Synechococcus* sp. have been shown in the

field to produce phytochelatins in response to copper stress (Moffett and Brand, 1996; Moffett et al., 1997). Phytochelatins are proteins that internally bind copper until it can be excreted. Both laboratory and field studies show that phytochelatins in organisms (and particles) increase in proportion to increasing copper stress. The binding strength of phytochelatins is consistently stronger (larger stability constants) than that for algal exudates. Indeed, copper binding agents have been described as belonging to two classes: a class of “strong” binding complexes with $K_{\text{Cond}} > 10^{12}$ and a weaker class with $K_{\text{Cond}} < 10^{12}$. In seawater media, in addition to complexing agents, the effect of copper may also be ameliorated by the presence of Mn(II) and Fe(II), which, when present in large quantities, may significantly reduce the toxic effects of Cu(II)_{aq} by directly competing for the same binding sites. Analogously to the production of phytochelatins, higher level organisms produce metallothioneins and higher molecular weight proteins that bind copper intracellularly and serve to regulate tissue copper levels by (ultimately) excreting the excess.

It is reasonable to assume that high levels of copper in the water or sediments also mean high levels of copper in marine organisms. Concomitantly, high levels of phytochelatins or metallothioneins in organisms also mean high water or sediment copper levels. VanderWeele (1996) showed that the mussel *Mytilus edulis* transplanted from a low-copper environment in Mission Bay (San Diego) to a high copper area of San Diego Bay, accumulated copper rapidly, and its tissue copper content was proportional to the ambient copper concentration of the water within a month. Those mussels placed in the areas of highest copper showed a reduction in growth rate over 6 months over those placed in cleaner areas. The condition index (weight/length) also reflected this trend.

DiBacco (1998) examined chronic lethal and sublethal effects of San Diego Bay versus southern California coastal habitats on the survivorship and development of crab larvae, *Pachygrapsus crassipes*. Body burden estimates of copper, which served as a proxy for bay contaminants, for newly hatched larvae, reflected their origin. Larvae originating from the inner half of San Diego Bay contained from three to eight times more copper than larvae from coastal waters. Regardless of their origin, however, these differences persisted no more than 2 days after hatching. Laboratory culturing experiments revealed that larvae reared in coastal seawater experienced significantly lower mortality than those cultured in San Diego Bay (figure 7). Larvae reared in coastal seawater had a significantly higher percentage of zoea surviving to the megalopal stage of development and successfully molting from the final larval stage (V) of development to the post-larval megalop than larvae reared in bay water. Megalopal duration (from molt until death) was greatest for zoea reared in coastal water as well as those brooded in coastal habitats. These results suggest that brood site and waters within which larvae develop can influence larval survivorship, development, and, probably, adult population dynamics.

(a) SWEETWATER MARSH, SAN DIEGO



(b) DIKE ROCK, LA JOLLA

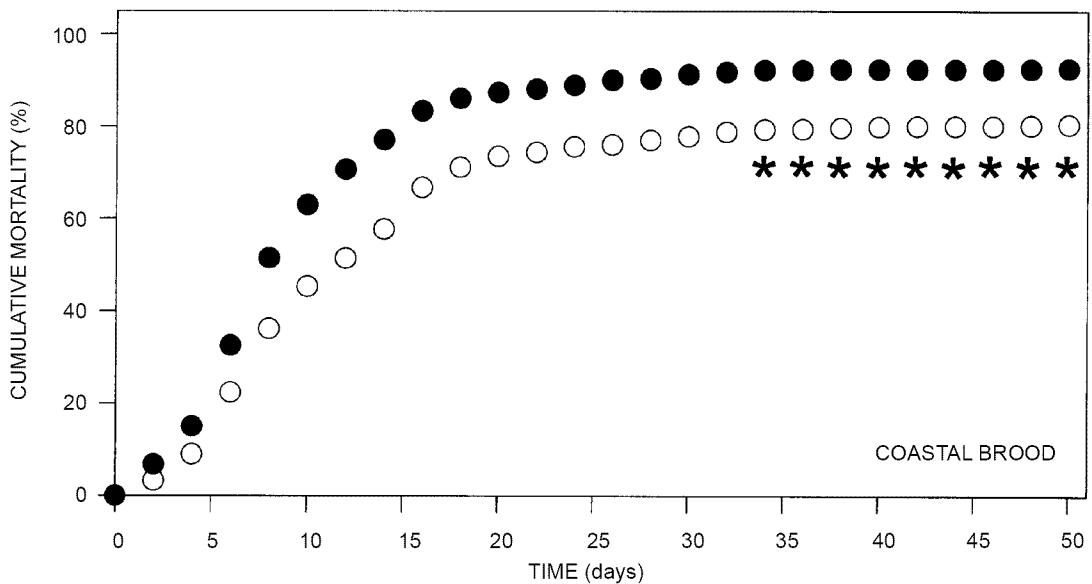


Figure 7. Culture water effects on laboratory cultured crab larvae, *Pachygrapsus crassipes*, expressed as percent cumulative mortality. Mean mortality estimates within each plot are independent of larval origin since larvae were brooded in (a) Sweetwater Marsh, San Diego Bay, CA, and (b) Dike Rock, La Jolla, CA. All curves are terminated at day 50, but cultures lasted an average of $98d \pm 6.61d$ (SD). Statistical differences (T-tests, $\alpha = 0.05$) in percent cumulative mortality estimates are indicated by asterisks (*) and persisted until cultures were terminated. Error bars are not shown for the sake of clarity.

COPPER BIOAVAILABILITY (J. Moffett, P. Paquin, and L. Mayer)

The two examples from San Diego Bay appear to suggest that the copper concentration is a good indicator of the copper that is taken up by organisms, while we have seen from figure 6 that it is the copper activity, expressed as pCu, that relates to toxicity, and not the total copper concentration. This apparent discrepancy deserves more discussion. The observed relationship between survival and metal activity is known collectively as the Free Ion Activity Model (FIAM). The FIAM hypothesis has been analyzed critically by Campbell (1995), and that review will be briefly summarized here for copper. Campbell (1995) presents examples, including marine algae, bacteria, molluscs, and crustaceans, where marine organismal toxicity has been found to conform to the FIAM. No saltwater fish are included, but from rainbow trout data, there is considerable evidence that some fish are also affected. Almost without exception, the determinations were made in controlled media such as filtered seawater to which copper and a complexing ligand had been added. Almost universally, the ligands were EDTA, nitrilotriacetic acid (NTA), and TRIS (tris(hydroxymethylaminomethane), ligands that form *hydrophilic* complexes to which biological membranes are rather impenetrable. Due to the fact that, in seawater, pH and alkalinity are relatively constant, these variables were not presumed to be important (and consequently not measured), contrary to the case in freshwater. There are apparent exceptions to the FIAM model. Principally, these are usually associated with copper complexed by *hydrophobic* or *lyophilic* organic ligands that are directly assimilated across cell wall and through the plasma membrane into the cell. Ethylxanthate, oxine, 8-hydroxyquinoline, and diethyldithiocarbamate are examples of lyophilic organic complexing agents used in copper testing that had the effect of enhancing copper toxicity over that predicted by pCu.

There is also some evidence of increased copper toxicity when copper is associated with low molecular weight, hydrophilic organic matter such as amino acids, citrate, or even ethylenediamine. In the tests, a dose-response curve is measured in a medium without the low molecular weight organic agent. The test is then repeated in the presence of the complexing agent, and, invariably, the toxicity of copper appears to have increased despite the fact that the complexing agent lowered the activity considerably. This again suggests a shortcut path for copper into the cell. For glycine and ethylenediamine, these results contradict the results obtained in figure 6, suggesting that these particular reported determinations may be organism-dependent or affected by other variables.

There is some question as to the effect of natural dissolved organic matter (DOM) on toxicity. Experiments with radioactive copper showed that the removal of hydrophobic DOM from estuarine water increased the particulate Cu/dissolved Cu ratio by a factor of two, suggesting that hydrophobic DOM was instrumental in complexing Cu and keeping it in solution (Paulson et al., 1994). Copper complexed in this manner might also be taken up directly by marine organisms.

In conclusion, while evidence for the FIAM hypothesis is strong, it does not necessarily exclude other copper species, and there may indeed be a special relationship between the toxicity of a particular species and a type of organism. Furthermore, the FIAM model has never been directly demonstrated in natural waters. Finally, copper species may be related to each other. In a recent study, Zirino et al. (1998b) showed that dissolved, colloidal, and acid-releasable copper in San Diego Bay samples were all statistically related to pCu, although the correlation was more significant in areas highly polluted with copper. In that work, Zirino et al. (1998b) concluded that more data from various marine environments needed to be collected before a truly general statement about the applicability of the FIAM model could be made. Nevertheless, their observation points to the fact that if any Cu species are to replace copper concentration as predictors of toxicity, they must also be shown to be *independent of the total copper concentration*.

Finally, all of the above presents a strong argument for the measurement of *both* pCu and dissolved, total recoverable, copper (Cu_T) in ecological studies. Their ratio would give a measure of the ability of the medium to absorb copper and render it nontoxic. Also, pCu and Cu_T may co-vary, thereby indicating rapid kinetics and possibly only a scale difference between the two. Alternatively, pCu may vary independently of Cu_T , indicating control of pCu by the medium and its buffering capacity.

The Gill Model

In the last decade, in experiments done in freshwater, it has been possible to correlate copper retained on fish gills (principally fathead minnow) with its toxicity to the same organism. Copper acts as a “surface active toxicant” and binds up sites on the gills that play critical roles in transport functions. Free $Cu(II)_{aq}$ appears to be the toxic species that impedes the normal uptake of Na^+ ion through the gill and causes potentially lethal decreases in plasma Na^+ ion (*hypocalcemia*). Different gill binding sites have been quantified and their affinity for copper has been characterized by measuring a “gill conditional stability constant” via a ligand competition technique. In this manner, Playle et al. (1993a, 1993b) were able to calculate a Cu-gill binding constant of 7.4, with 2 nM of binding sites per fish. In essence, the gill itself can be modeled as another ligand in an equilibrium system of solution (seawater) ligands that compete for the free ion. If the solution (water) ligands sequester the free copper (have $K_{Cond} > 7.4$ and offer more binding sites), then there is virtually no $Cu(II)_{aq}$ available to the gill, and the water is much less toxic to the fish.

The key point here is that one can predictively model the acute response of the organism based on the solution chemistry, because mortality is directly related to the amount of Cu bound to the gill copper sites. For chronic exposures, additional considerations need to be considered, including detoxification by sequestering the copper with metallothioneins, incorporating copper in the insoluble granules, etc. It is important to realize that “Modeling metal binding to gills of fish directly considers the influence of water chemistry on metal binding at the target organ of acute metal exposures in fish. This approach explicitly links water chemistry, metal concentration, and the organism” (Wood et al., 1997). In effect, the gill model applies the FIAM to the test fish.

There is, however, an important distinction between the gill model and the FIAM. The gill model quantifies competitive effects of Cu with other cations, such as Ca^{++} and Na^+ , such that toxicity is not necessarily related directly to copper activity, as it is in the FIAM. This effect is illustrated in figure 8, which shows fathead minnow LC50 data (Erickson et al., 1996) as a function of Ca^{++} , with LC50's expressed in terms of total Cu concentration, free Cu, and gill Cu, on the upper, middle, and lower panels, respectively. As shown on the upper panel, the measured total Cu LC50 increases with increasing Ca^{++} , a trend that is consistent with the current WQC for copper, which increases as a function of hardness as well. The middle panel displays the free Cu LC50's, calculated from the measured total copper LC50's (upper panel), and they also increase with increasing Ca^{++} , indicating that the measured effect, 50% mortality, is not uniquely associated with a single free copper level. This result is clearly not in conformity with the FIAM. In contrast to the free copper results, the calculated gill Cu accumulation (bottom panel) is relatively consistent over the range of calcium concentrations tested, averaging about 12 nM/g_w. The reason the gill copper LC50 is relatively constant even as the free copper LC50 increases is that the calcium competes with the free copper in solution for binding at the site of action. Hence, a higher free copper level is required to achieve the same associated effect (i.e., 50% mortality), as calcium increases.

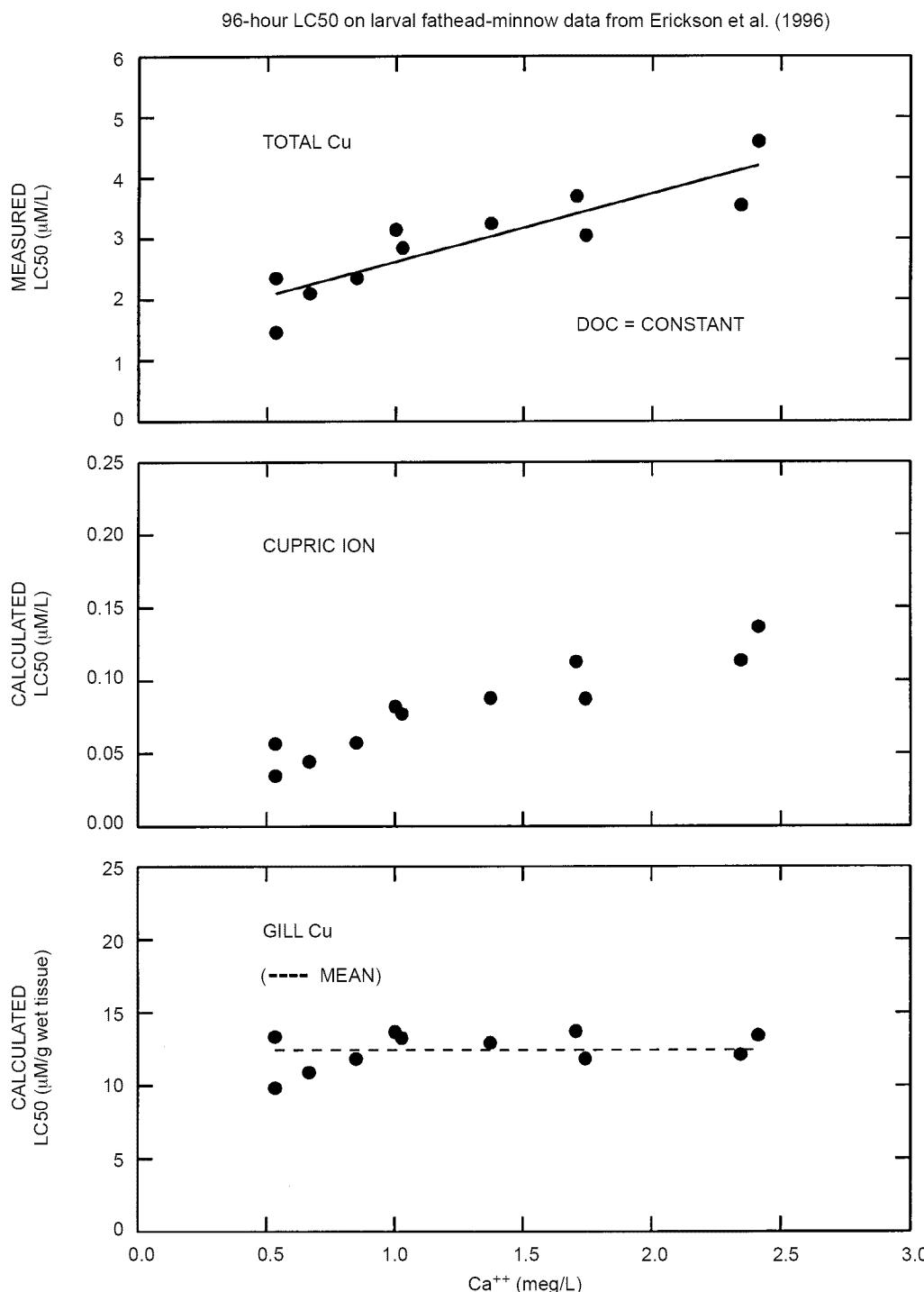


Figure 8. Fathead minnow LC50 data as a function of Ca⁺⁺ concentration.

At the workshop, it was proposed to attempt to extend this approach to estuarine waters. The task is facilitated by the fact that the composition of seawater is much more predictable than the concentration of freshwaters. However, as of yet, there is no database available that uses a saltwater fish, although this is probably not a serious obstacle. Two questions that remain are (1) how does one

extend this system to invertebrates, and (2) how do other methods of exposure, such as direct ingestion and adsorption, contribute to toxicity?

Cu Bioavailability In Sediments

Differences in chemical speciation appear to be instrumental in determining toxicity in sediments as well as in the water column. Figure 9 shows the distribution of effect and no effect data in freshwater sediments used to derive the Canadian sediment quality criteria (Allen, 1996). It is quite obvious from the figure that the values set for the threshold effect level (TEL), circa 36 mg/kg, are fairly accurate in predicting that a toxic effect is not likely to be observed with a sediment sample whose copper concentration is below this value. Unfortunately, there is only a 50% chance of predicting whether a sediment is toxic if its copper content is *above* the TEL level, *regardless* of its concentration! The reason for this is that organisms do not necessarily respond to bulk concentration, but respond instead to the bioavailable portion of the total copper concentration that is related to the “sediment extractable metals” or SEM of the sample. If copper in the sediment is being sequestered in an insoluble form, then it is not likely to be toxic. Drawing upon measurements of cadmium in marine waters (no suitable copper data being available), it was pointed out that the toxicity of copper in marine sediments was probably related to the ratio of sediment-extractable copper to excess acid-volatile-sulfides (AVS) present. An excess of AVS would bind copper in an insoluble sulfide and render it nonbio-available. Otherwise, the copper could become available (and toxic) through the actions of marine organisms on the sediment.

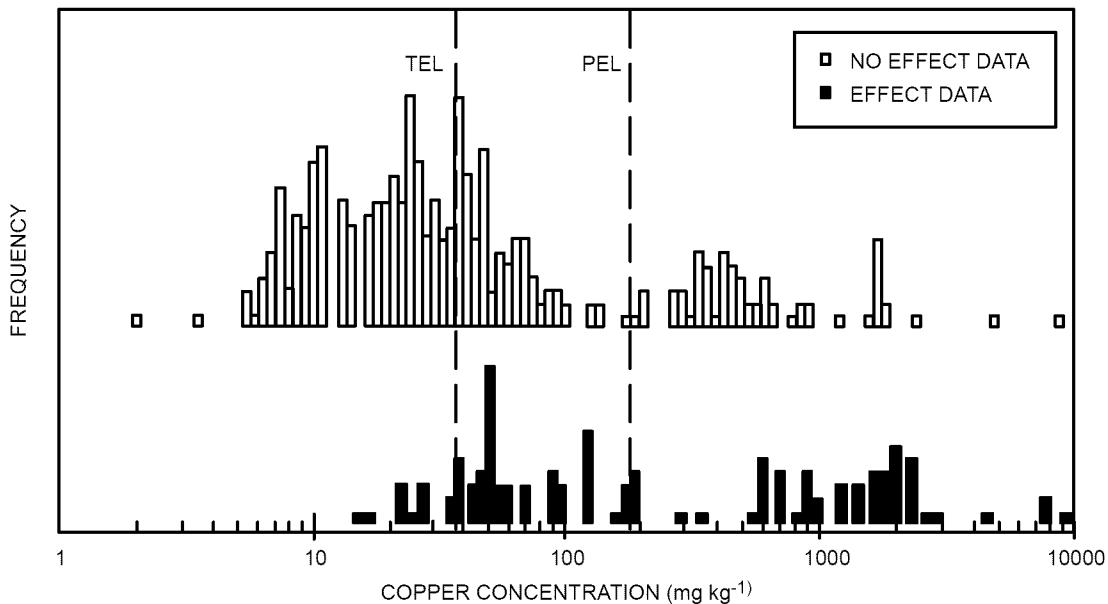


Figure 9. Distribution of effect and no effect data in freshwater sediments used to derive the Sediment Quality Guidelines (data after Smith et al., 1996).

Polychaetes and holothurians (worms and sea cucumbers) are among the major agents that rework estuarine sediments by passing them through their alimentary canal and digesting the organic material in the process. The once-processed organic matter that is not digested in the gut of the organisms is then expelled as fecal material. This process lowers the pH of the interstitial water and consumes oxygen. On average, a concentration of about 1 to 5% of organic carbon remains in the sediment to

become buried. Mayer and colleagues (Mayer et al., 1996; Chen and Mayer, 1998) have made a study of the remobilization of copper in the digestive (gut) fluids of various species of annelids (polychaetes) and echinoderms (holothurians) and observed a close correlation between the copper content of the fluid and the amino-acid concentration. They also observed a gradient in enzyme activity and dissolved proteins between the mouth and anus of the organisms. The highest concentration of metal activity was, reasonably, in the midgut of the animals.

When the digestive fluid was fractionated by molecular weight and analyzed for total copper, a close correlation emerged between the quantity of amino acids in each fraction and its copper content, suggesting a functional relationship between the two. Another interesting observation made by Mayer and colleagues is that the digestive fluids release copper from the sediments by chelation rather than by enzyme action. This process was demonstrated by inactivating the enzyme action using microwave heating, leaving behind only the complexation capability of the proteinaceous materials. Using a chemical blocking approach, histidine was found to be the amino acid responsible for most of the complexation capability of digestive peptides. Histidine is well known as a strong copper complexing agent in many proteins. It was found that significant copper was released by digestive attack only if the 1N HCl-soluble fraction of Cu was in the 1 to 2 $\mu\text{M/g}$ excess of the acid volatile sulfide concentration. This finding is consistent with the findings of the SEM-AVS technique. This approach to the study of bioavailable copper in sediments is providing both a mechanistic understanding of Cu mobilization and toxicity and a basis for the design of artificial cocktails that can be used routinely to measure bioavailable Cu in marine systems.

THE EFFECTS OF COPPER ON MARINE ORGANISMS (S. N. Luoma, S. Steinert, R. Streib-Montee, and P. Weis)

In nature, it is difficult to attribute adverse effects of copper contamination directly to organisms. This is due to a rather large number of factors, such as the difficulty of isolating the effects of copper from those of other contaminants. Similarly, it is difficult to assess how toxic effects at one level of biological organization affect processes at the next higher organizational level. Direct evidence of bioaccumulation is also not proof of toxicity. For long-term studies at low levels (studies that best mimic nature), detoxifying mechanisms in some organisms may allow them to survive and actually thrive under conditions lethal to others. Some of the complex interactions that can occur in a marine ecosystem when copper (or any other toxin) is added to the environment are shown in figure 10.

For the reasons mentioned previously, conclusive proof of copper toxicity is rare. However, a long-term study in San Francisco Bay may have successfully related copper (and silver) contaminated sediments from a mud flat in Palo Alto (at the southern end of the bay) to stress in the resident clam *Macoma balthica*. Contamination originated from the effluent of a sewage treatment plant and from sediments in the vicinity of the plant. Copper (and silver) levels in the vicinity of the plant were monitored for 17 years. From 1977 to 1993, extractable sediment copper was between 30 and 60 mg/kg, leveling out thereafter to about 20 to 30 mg/kg. Tissue copper also showed elevated values over this time period, ranging from about 100 to 300 $\mu\text{g/kg}$, leveling out to about 70 $\mu\text{g/kg}$ after 1987. Biochemical symptoms of stress were identified by analysis of the clam tissue. Physiological stress was indicated by reproductive anomalies, including a reduction in the percentage of reproductive clams, which correlated to the annual maximum Cu concentration of the tissues and an inordinately high percentage of males in the population. The clam population at the contaminated site was also found to be six times more resistant to Cu than populations from noncontaminated sites. For this site, “*the combined chemical, biochemical, physiological, and life history studies provided a preponderance of evidence describing metal effects to at least the population level*” (Luoma, 1995).

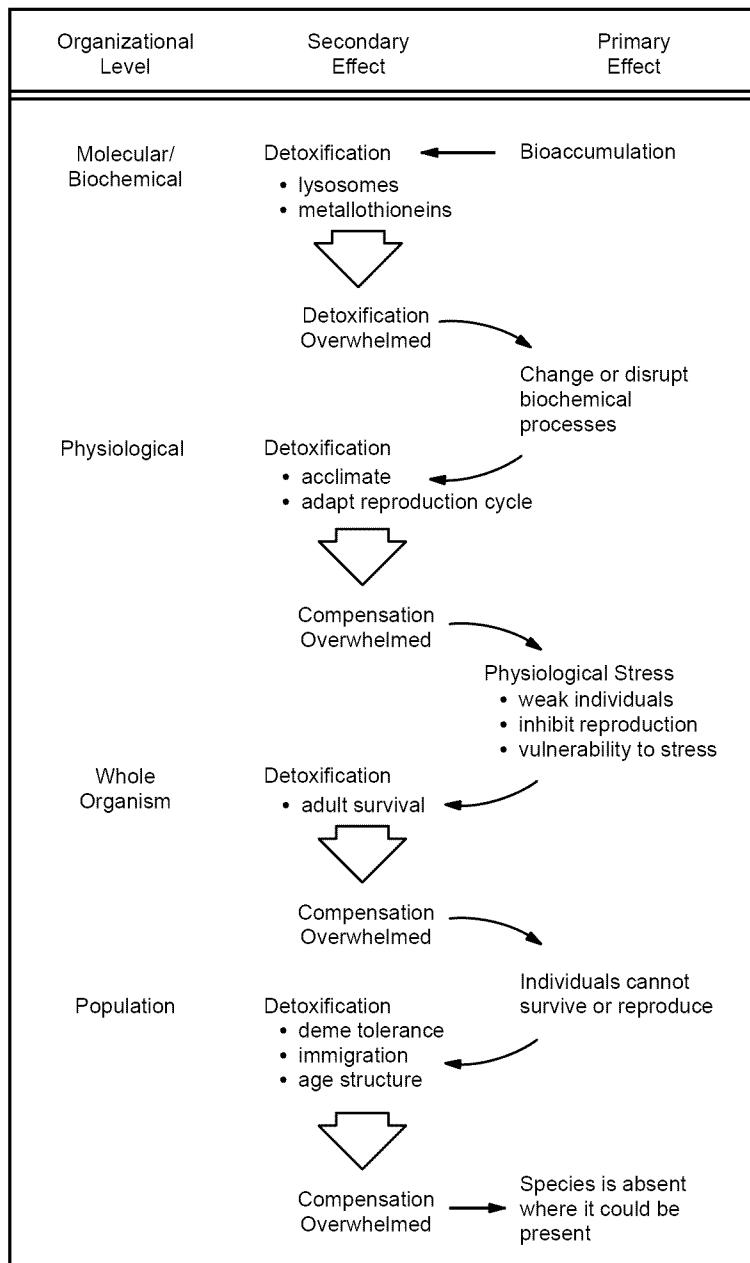


Figure 10. Cascade of effects from the bio-chemical level of organization to the population level. The continuum of effects defines the toxicity process in nature (after Luoma, 1995).

Assessing DNA Damage—The Comet Assay

The identification and characterization of Cu-impacted sites is an ongoing priority for the Navy. While no one bioassay method is sufficient on its own to directly link Cu impact to *organismal* toxicity (see previous section), the Comet assay is capable of detecting stress-induced cellular DNA damage, which is a sensitive indicator of toxicity in all organisms. The method is versatile, cost effective, and requires only basic equipment found in most biologically oriented laboratories.

Sample preparation for the assay requires as few as 5,000 cells suspended in agarose and applied to a microscope slide. The immobilized DNA is denatured under alkaline conditions, and broken or relaxed strands of DNA are allowed to migrate away from the nuclei in an electric field. The slides are then stained with a fluorescent nucleic acid stain and viewed with an epifluorescence microscope. The distance and/or amount of DNA migration from individual nuclei are indicative of the number of strand breaks. Using the technique, one can determine increases in DNA damage, determine DNA repair activity, identify specific types of DNA alterations (damage), and determine increases in cell turnover rates. Using image analysis software, it is possible to distinguish different cell types by their DNA content. This allows the differentiation of germ cells from somatic cells and allows the identification of somatic cells at different stages of the cell cycle. In this way, cells with vastly different sensitivities to and capabilities for handling DNA damage can be examined separately from one another.

The Comet assay has been used to measure the effects of environmental contaminants in San Diego Bay (Steinert et al., 1998). Mussels were deployed 1 meter off the bottom, under ambient conditions for 32 days, at both “pristine” and contaminated sites. At the end of the exposure period, hemolymph collected from the exposed organisms was analyzed using the Comet assay, and whole mussel tissues were analyzed for tissue contaminant loads. Animals deployed at the “pristine” reference site showed the least amount of DNA damage and the highest growth rates. Animals exposed to high copper at known contaminated sites had significantly lower growth and the highest levels of DNA damage. Figure 11 shows the relationship between copper bioaccumulation in mussel tissue and levels of DNA damage in the bay mussel *Mytilus edulis*. Since copper concentrations in the water column were uniformly low, it is likely that this tissue burden was caused by uptake of contaminated sediments resuspended by ship traffic.

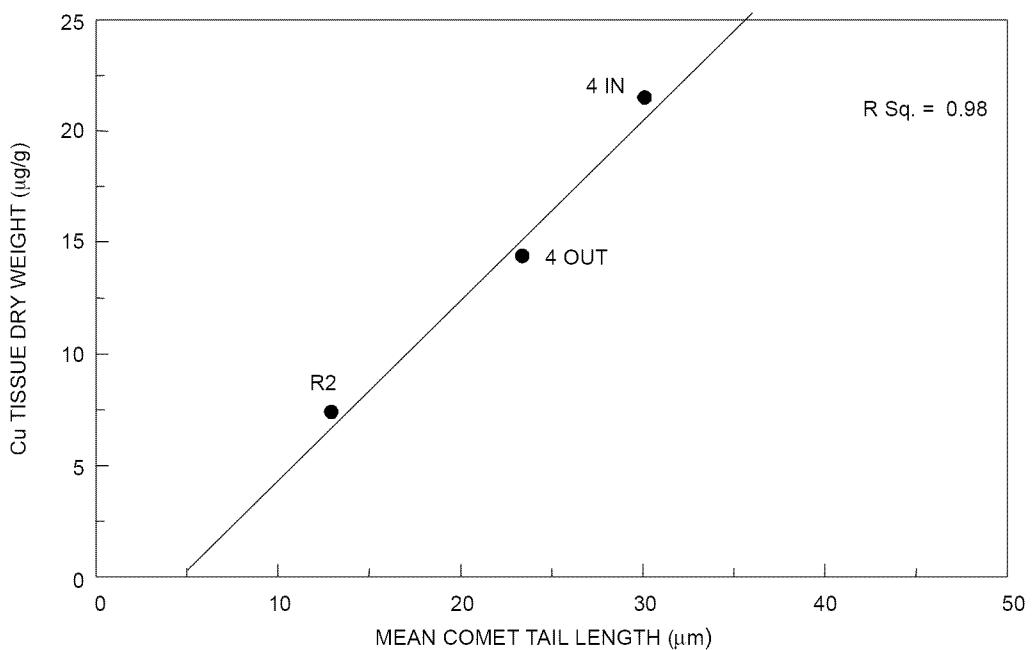


Figure 11. Relationship between “comet” tail length and copper in *Mytilus edulis* tissue at three stations in San Diego Bay.

Copper Toxicity in Fish

The toxicity of Cu to aquatic organisms is well established, and in fish, it is no different than in most other taxa. Among the more common metal pollutants, Cu is exceeded in toxicity only by silver and mercury. The early life stages of fish are especially vulnerable, with the early gastrulation stage being the most sensitive embryonic stage. In many species, the embryo receives some protection from its chorion (the avascular shell-like membrane that surrounds fish embryos) so that, after hatching, the larvae may die in the same test solution that was resisted by the embryos. Once the liver has developed, the metal binding protein metallothionein (MT) is synthesized, and thus, heavy metals burden, including Cu, can be managed. However, the limits of MT synthesis and function may be exceeded, with the excess Cu “spilling over” into and binding to the cytoplasmic enzyme pool. Enzyme inhibition is a principal cause of copper toxicity.

USER AND REGULATORY GROUP WRAP-UP SESSION AND RECOMMENDATIONS (Facilitator: P. F. Seligman)

A number of issues and problem areas regarding copper discharges and their impact on the receiving system were defined and discussed by the group.

COPPER DISCHARGES OF CONCERN

Discharges from dry docks at some naval shipyards periodically exceed discharge limits. Discharges from ship holding tanks to onshore Public Owned Treatment Works have occasionally exceeded copper limits. Stormwater (nonpoint source) discharges are often a major source of copper and are probably the largest source in some regions. Industrial waste pretreatment and other point sources can also be high in copper. Sources from ships include releases from antifouling coatings that are probably the largest mass discharge of copper into high-use harbors and estuaries and may come under increased regulatory scrutiny. Discharges from in-water hull cleaning and hull husbandry, although relatively infrequent, can produce a high copper concentration effluent. Ship discharges such as cooling water, fire mains, bilge discharge, gray water, etc., are also being evaluated for copper mass loading under the UNDS development process.

SPECIFIC REGULATORY ISSUES

The form of copper that is being regulated is not highly relevant to environmental effects. Cu(II)_{aq} is believed to be the most available and toxic species while total recoverable copper is not closely related to toxic response. Water quality criteria based on the latter are being used to define NPDES permit limits. A better approach is needed to understand mass loading parameters for the watershed and to develop total maximum daily loading criteria from which to derive the appropriate waste load allocations. A prerequisite to developing waste load allocations is a more complete understanding of the impact of the discharge on the receiving system. Sediment copper limits need to be developed, probably based on some measure of bioavailability rather than a simple bulk sediment concentration. Over the next several years, the Navy, in partnership with the U.S. EPA, will have to define copper (and other) standards under the UNDS process for ship discharges. These discharge limits will be based on practicability and cost, as well as environmental effects, and therefore, may not mirror NPDES limits. However, their discharges will need to be considered for waste load allocations in the future.

IMPORTANT QUESTIONS TO BE ADDRESSED

The Navy user/regulatory group listed the following as the most important questions that need to be addressed:

1. What are the forms or species of copper that are most bioavailable or of most importance in the environment, and can standards be developed for them?
2. At what concentration is copper safe to discharge?
3. Can reasonable, but scientifically sound and defensible standards (or targets) be established for the purpose of infrastructure and pollution control planning for Naval Facilities, Shipyards, Naval Stations, UNDS, etc.?

POLLUTION CONTROL

There is need to balance pretreatment and treatment controls in a cost-effective manner. Both the Navy and industry need faster, cheaper, and better biological and/or chemical treatment methods to remove copper. Faster regulatory acceptance is needed for both measurement and pollution control techniques to support systems that can be engineered to meet reasonable and scientifically sound standards at achievable cost. New in-water hull cleaning technology needs to be developed to reduce production of copper waste and/or enable collection of discharge water for treatment. Industrial waste treatment plants need low-cost removal systems for copper (and other metals) and better analytical approaches to monitor metal output. New treatment options are needed to reduce dry-dock copper discharges from sources such as 2000 psi wash water, overspray, dust from sand blasting, and runoff from rain.

UNDS PROCESS

By February 2000, the UNDS process will need to develop discharge standards for selected marine pollution control devices (MPCDs). It is important to determine what sampling and what measurement methods should be done in the near future to obtain the information necessary to develop those standards with the concurrence of EPA. Issues that will have to be resolved under UNDS include how to develop a single achievable standard that is protective of the majority of the harbors and coastal regions in which the Navy operates. Complex questions that will have to be dealt with include deciding whether copper antifouling coatings will need an MPCD and how it would be implemented. Perhaps even more difficult is the question of how to handle cooling water copper loading. Other questions that the group asked were how would copper limits be derived, and how does that relate to mass loading from the ship as a whole? How should we be measuring and reporting copper relative to the derived limits? Should we be measuring copper activity and/or other species? To what degree should ecological risk assessment be used to support derivation of the standard(s)? Do current levels of copper in the environment have a negative impact on the biota? The latter was perhaps the hardest question to answer.

HOW CAN SCIENCE HELP?

Several suggestions were made on key scientific issues or questions that need to be addressed. These were (1) that acceptable and relevant measurement tools be provided to support appropriate reasonable water quality objectives and MPCD performance standards, (2) that the time scales of Cu complexation be determined and that these rates be related to toxicity criteria, (3) that nontoxic or reduced release rate coatings be developed, and (4) that the engineering implications of Mn and Fe as complexing ions in reducing toxicity be explored.

WORKSHOP SCIENTIFIC RECOMMENDATIONS (Facilitator: A. Zirino)

One of the objectives of the workshop was to recommend specific research areas in which development would improve our understanding of the relationship between the input of low levels of copper into an estuarine environment and its ultimate ecological consequences. For this purpose, after the individual presentations, the invited speakers and their scientific audience were asked to participate in an open, informal discussion on this topic. Understandably, and in keeping with the interdisciplinary aspects of the problem, several areas were identified. These are summarized below.

1. Integrate bio-geochemical aspects of copper into hydrodynamic models

At the workshop, there was a consensus that environmental copper toxicity was tied to copper speciation, and there was also agreement that it is essential to be able to predict the distribution of copper species in an estuarine environment from the point of introduction to final destination out of the system. To do this, however, one has to have a clear operational definition of the species in question. Secondly, the rates of *in situ* species formation and transformation should be known. In a dynamic environment such as an estuary, where the dispersion of copper is dependent upon tidal mixing, wind mixing, and transport of particulates, the dynamics of copper can only be followed by coupling the copper geochemistry to a complete hydrodynamic model. Since all of the copper is considered to be partitioned between dissolved and colloidal organic matter and organic matter on clays, it becomes patently clear that the coupling must be done by introducing the carbon cycle of the estuary into the hydrodynamic model. This, in turn, involves a knowledge of the rates of photosynthesis and respiration of the estuarine environment. Special attention must be given to the role of sediments as microbially active sources and sinks of copper. Once the coupling has been done and a satisfactory knowledge of the steady-state distribution of copper among various species has been obtained, it then becomes possible to address the toxicological questions.

2. Standardize analytical protocols for copper species and copper complexation

Several important and distinct copper species were discussed and identified during the workshop. These included the hydrated copper ion, Cu(II)_{aq}, inorganic copper complexes, copper complexed with dissolved organic ligands, copper associated with organic colloids, copper adsorbed on clays, etc. Some or all of these species may have a distinct functional relationship to toxicity. However, at present, there is no standardized protocol for either distinguishing or measuring them. The measurements of total recoverable copper, copper ion activity, and the copper complexation capacity of the water, (all of major environmental importance) have also not been standardized. There exists a need to develop standardized methods for copper speciation and complexation capacity.

3. Study the role of organic matter in ameliorating the environment

This is a complex area that interlaces physical environment, productivity, respiration, and the physical nature of the organic carbon produced. Indeed, it is a subset of item 1 above. Products of microalgal and microbial activity that are able to strongly bind copper and control its partitioning between the dissolved, colloidal, and particulate phases need to be identified and adequately characterized. The present practice of assigning operational binding constants to a large class of Lewis bases is an insufficient characterization of the binding system because it does not address its physical properties and needs to be improved. Also, it is important to know how other metals besides copper are also bound in the medium because the availability of other metals, such as Zn, Fe, and Mn may influence the toxicity of copper. The special role of sediments and sedimentary organisms in deter-

mining the micro-chemical composition of the water needs to be emphasized. Factors that affect the sedimentary fluxes of copper and metal-binding organic matter also need to be determined.

RELATION BETWEEN COPPER TOXICITY AND STRESS TO ORGANISMS

Because the speciation of copper is considered an important factor in the determination of toxicity, some EPA-approved toxicity tests presently used may need to be reevaluated in terms of chemical speciation. This is especially true for very sensitive organisms tested for acute toxicity by using "nominal" copper additions. In the tests, these organisms were subjected to high concentrations of "free" copper (such as CuSO₄), under conditions very likely to be dissimilar to those in the natural environment. Since it is these tests that essentially "drive" the WQC, they ought to be repeated using the latest analytical techniques for measuring copper speciation.

It is important to be able to translate accurately the effects measured in individuals to population and community effects. This is also true when translating the results from standard bioassays to population and community effects in nature. However, given the complexities of the natural environment and large differences in the reactions of organisms, sometimes even within the same biological species, such extrapolations are rarely successful at low levels of copper. Much more work needs to be done to understand the actual mechanisms of the toxic reactions and of the interactions among populations and communities.

It is also quite obvious that our knowledge of the interactions of natural communities in the environment is quite limited. This is because, at present, taking a census of the biological communities of even a small estuary is an impossibly laborious task, never carried out to completion. Thus, it is recommended that, in the future, more effort be devoted to the development of improved census techniques, such as underwater video imaging, which, with adequate image processing, would allow the estimation of the size of at least some populations over large areas. If these types of instrumented surveys were coupled to similarly automated, *in situ*, measurements of hydrographical and key chemical elements (such as copper species), a much more complete knowledge of the chemical-biological interactions in the environment would result.

Finally, techniques that indicate stress in individual organisms in the natural environment (such as the Comet assay for DNA damage) should be developed further. While it is difficult to relate such evidence to an individual component of seawater such as copper, lack of stress is also an indication of low toxicity, regardless of concentration. When stress is present, specific biochemical signatures may permit the stress from "natural" causes to be distinguished from that of possible anthropogenic origin.

CONCLUSIONS

1. The legal release and disposal of copper or copper-laden effluents into estuarine waters poses a serious environmental, engineering, and economic challenge to the Navy and the country in general. This is because EPA-mandated WQC for copper effluents are at, or very close to, the ambient or “normal” concentrations of copper in many estuaries, which are at the low $\mu\text{g/L}$ level. The treatment of copper-contaminated effluents to achieve such low levels, even if this is technologically attainable, may be cost-prohibitive and may not be warranted since there is little, if any, direct evidence in the natural environment of toxicity caused by small increases in copper levels. The issue, however, is scientifically complex because effluents are dispersed; copper is complexed and rendered less toxic by natural organic matter; and resilient organisms adapt and fill niches vacated by more sensitive species. The issue is also beyond just a purely scientific question since it ultimately involves a value judgment as to what constitutes a baseline level and the degree of environmental alteration society is willing to accept in exchange for technological and economic benefits.
2. The present EPA-mandated WQC justifiably fulfill the mission of protecting the environment but do so from a relatively weak scientific basis and, most probably, are over-protective. Such is the case because the WQC were obtained in laboratory tests while using pristine waters. Criteria were based on total concentrations without any concern for copper speciation, which overwhelming scientific evidence shows to be instrumental in determining toxicity. The dichotomy between current scientific knowledge and WQC standards tends to undermine confidence in the general applicability of the standards.
3. At present, a large body of scientific data indicates that it is the concentration of the “free” or aqueous cupric ion species ($\text{Cu(II)}_{\text{aq}}$) that relates best, but not exclusively, to the toxicity of marine organisms. There is a good scientific basis for this, and it goes beyond the notion of a small quantity of copper (< 1% of total Cu) being very toxic. *The activity or concentration of $\text{Cu(II)}_{\text{aq}}$ is a good measure of the lability and mobility of all the copper in the system and, therefore, would be expected to be highly correlated to its uptake by organisms and to its ultimate toxicity.*
4. Current trends in metal water quality criteria indirectly reflect current knowledge about the importance of speciation through a number of mechanisms, such as the adaptation of “dissolved” copper concentrations rather than total recoverable copper and through the option of using WERs that account, in part, for the $\text{Cu(II)}_{\text{aq}}$ sequestering capacity of dissolved organic and colloidal materials found in natural waters. However, such measures do not take into account the temporal dynamics of $\text{Cu(II)}_{\text{aq}}$ and, in reality, cannot be substituted for the direct knowledge of the dynamics of $\text{Cu(II)}_{\text{aq}}$.
5. Indeed, there is evidence in the literature that the ratio of free copper to total dissolved copper ($\text{Cu(II)}_{\text{aq}}/\text{Cu}_T$) may vary both temporally and spatially due to local mixing processes, adsorption on particulates, and the ability of marine microorganisms to produce copper-sequestering materials (Muller, 1996; Moffett et al., 1997; and many others). It is also likely that the conditions under which standard toxicity tests are performed do not reflect accurately the $\text{Cu(II)}_{\text{aq}}/\text{Cu}_T$ ratio found in the natural environment. Therefore, in order to better relate WQC to natural conditions, a concerted effort should be made to understand how $\text{Cu(II)}_{\text{aq}}/\text{Cu}_T$ changes in the natural environment as well as how $\text{Cu(II)}_{\text{aq}}/\text{Cu}_T$ changes over time during toxicity tests.

6. Analytical techniques for the measurement of (at least) two of the many possible copper species, $\text{Cu(II)}_{\text{aq}}$ and Cu_T , should be standardized, and “trace metal clean” techniques should be used in both sampling and measurement so as to provide unambiguous reference levels for the “user” community.

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